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(54) Title: SCREENING METHOD FOR MENA PROTEIN INVOLVED IN MICROFILAMENT DYNAMICS

(57) Abstract

Two novel mammalina genes *Mammalian Ena (Mena)*, and *Ena-VASP like (Evl)* encoding novel proteins Mena, and Evl are disclosed. Mena and Evl proteins have a discrete, EVH1 functional domain responsible for Mena binding to ActA of *Listeria*, and to the cytoskeletal proteins zyxin and vinculin. The EVH1 domain of Mena is also responsible and sufficient for targeting localization of Mena and Mena based fusion proteins to focal adhesions, and to the surface of *Listeria* cells at the polar site of induction of actin comet tail formation. Based on the Mena and Evl genes and proteins disclosed herein, a variety methods and compositions are provided for screening, isolating, and characterizing endogenous and exogenous factors, drugs and therapeutic agents useful to evaluate and/or control cytoskeletal dynamic events involved in normal and abnormal cell morphology, adhesion, motility, growth and/or differentiation.

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NOVEL DNA SEQUENCES ENCODING PROTEINS INVOLVED IN MICROFILAMENT DYNAMICS

Government Support:

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Technical field:

The invention relates to DNA sequences encoding proteins involved in microfilament dynamics. More specifically, the invention relates to DNA sequences and proteins involved in microfilament dynamics affecting normal and abnormal cell morphology, adhesion, motility, growth and differentiation, as well as pathogenicity of certain viruses and bacteria, and to screening, diagnostic and therapeutic methods and compositions employing these DNA sequences and proteins.

BACKGROUND OF THE INVENTION

The control of cell morphology, motility, growth and differentiation generally requires coupling of external stimuli to processes that regulate cytoskeletal architecture. The mechanical forces that drive morphological change and migration arise initially from the microfilament-based cytoskeleton (reviewed by Lauffenburger and Horwitz, *Cell* 84: 359-369, 1996; Mitchison and Cramer, *Cell* 84: 371-379, 1996). In particular, formation of cellular protrusions such as filopodia and lammellipodia requires polymerization and stabilization of F-actin. A growing body of evidence links various signal transduction pathways to the regulation of these cytoskeletal processes (reviewed by Zigmond, *Current Opinion in Cell Biology* 8: 66-73, 1996), but the final

integration of these signals with regulation of *de novo* actin polymerization is a complicated process that remains to be elucidated.

Actin-driven formation of membrane protrusions is the first step in migration of neuronal growth cones (Forscher *et al.*, *Nature* 357:, 515-8, 1992; Forscher and Smith, S., *J. Cell Biol.* 107:1505-1516, 1988; Sanders and Wang, *J. Cell Sci.* 100:771-780, 1991). Establishment of proper connections in the central nervous system depends on the ability of neuronal growth cones to guide neurites to their final targets. Genetic analyses have been used to probe the relationship between signal transduction pathways and neuronal development.

One signaling pathway implicated in the processes of axonal outgrowth in neural development is mediated by the Drosophila homolog of the c-Abl tyrosine kinase (Gertler, *Thesis*, University of Wisconsin-Madison, 1992; Gertler *et al.*, *Cell* 58:103-113, 1989; Gertler *et al.*, *Science* 248:857-60, 1990; Gertler *et al.*, *Genes Dev.* 7:441-453, 1993; Henkemeyer *et al.*, *Cell* 63:949-960, 1990). Simple *Abl* mutant animals survive past metamorphosis. However, *Abl* mutants that are also heterozygous for a mutation in *Disabled (Dab)*, or one of 4 other loci identified in genetic modifier screens, require Abl tyrosine kinase activity for post-pupal viability and proper formation of the embryonic central nervous system (CNS). Animals that are homozygous mutant for both *Abl* and *Dab* make few or no proper axonal connections.

The defects caused by loss of Abl, Dab or both are ameliorated by mutations in the Drosophila *Enabled (Ena)* gene (Gertler *et al.*, *Genes Dev.* 9:521-33, 1995; Gertler *et al.*, *Science* 248:857-60, 1990). *Ena* was the only locus recovered in repeated genetic screens for dose-dependent suppressors of *Abl*- and *Dab*- dependent phenotypes. In the homozygous state, *Ena* mutations cause a recessive lethal phenotype that includes defects in the embryonic CNS. Ena protein is concentrated in CNS axons, like Abl and Dab, and has a proline-rich core which binds *in vitro* to the SH3 domains of Abl and Src, but not to the SH3 domains of several other proteins tested (Gertler *et al.*, *Genes Dev.* 9:521-33, 1995). Ena is tyrosine

phosphorylated *in vivo*. The phosphotyrosine content of Ena is reduced approximately three-fold in *Abl* mutant pupae, indicating that Ena is phosphorylated by Abl-dependent and independent tyrosine kinases. Over expression and *in vitro* phosphorylation studies demonstrate that Abl can phosphorylate Ena directly. However, Abl-mediated phosphorylation of Ena is not absolutely required for axonogenesis, as the need for Abl tyrosine kinase activity in this process is exposed only in combination with mutations in sensitizing loci such as *Dab* (Henkemeyer *et al.*, *Cell* 63:949-960, 1990).

While the above findings implicate Ena as an important factor in Abl mediated regulation of axonal development, more specific knowledge is required to fully elucidate the function and regulation of Ena in axonogenesis and cytoskeletal dynamics in Drosophila and other organisms.

In addition to the neural development model of Abl-mediated cytoskeletal regulation, a second model for studying microfilament assembly and function has been developed based on certain intracellular bacteria that recruit host cytoskeletal proteins to achieve bacterial motility. The cytoskeletal dynamics involved in intracellular movement of these bacteria bears some resemblance to the actin dynamics in lamellipodia of motile eukaryotic cells (Theriot *et al.*, *Trends Cell Biol.* 2:219-222, 1992), suggesting that these model systems may be useful for determining the molecular bases of actin-based cell motility. The most widely studied motile bacteria in this context is *Listeria monocytogenes*, an ubiquitous Gram-positive bacterium responsible for severe, food born infections in humans and animals. *Listeria* motility and intercellular infection depends on the asymmetric recruitment of host profilin, an actin monomer binding protein, to one pole of the bacteria where rapid polymerization of actin is induced to form a microfilamentous "comet tail" that drives bacterial movement (Theriot *et al.*, *Cell* 76:505-517, 1994).

The interaction of profilin with the surface of Listeria is indirect and requires a bacterial gene *actA* (Chakraborty *et al.*, *EMBO J.* 14:1314-1321, 1995). The product

of the *actA* gene is a surface bound polypeptide, and is the sole bacterial factor needed for the inducement of host actin polymerization (*Id.*). However, recent findings have shown that *Listeria* grown in culture does not associate efficiently with either actin (Tilney and Tilney, *Trends Microbiol.* 1:25-31, 1993) or profilin (Theriot *et al.*, *Cell* 76:505-517, 1994, suggesting that recruitment of these proteins also requires a host cytoplasmic factor.

Recent observations implicate the Vasodilator-Stimulated Phosphoprotein (VASP) as a candidate factor involved in ordinary cytoskeletal dynamics, and as a host factor in the regulation of *Listeria* induced microfilament assembly. Specifically, the VASP protein is an abundant *in vivo* substrate for cyclic-nucleotide dependent kinases (Halbrügge *et al.*, *J. Biol. Chem.* 265:3088-3093, 1990; Halbrügge and Walter, *J. Chromatogr.* 521:335-343, 1990; Halbrügge and Walter, *Eur. J. Biochem.* 185:41-50, 1989; Waldmann *et al.*, *Eur. J. Biochem* 167:441-448, 1987). VASP is distributed along microfilaments, is particularly concentrated at sites of focal contact and in the peripheral lammelli of spreading or migrating cells, and there is evidence of direct contact between VASP and F-actin (Reinhard *et al.*, *EMBO J.* 11:2063-2070, 1992; Haffner *et al.*, *EMBO J.* 14:19-27, 1995). VASP also contains a central region of proline-rich sequences comprising a proposed binding domain for the actin binding protein profilin (Reinhard *et al.*, *EMBO J.* 14:1583-1589, 1995). In *Listeria* infected cells, VASP is recruited to the bacterial surface by direct binding with the ActA surface protein (Chakraborty *et al.*, *EMBO J.* 14:1314-1321, 1995; Pistor *et al.*, Curr. Biol. 5:517-525, 1995). VASP accumulates on the bacterial surface prior to the formation of F-actin clouds that precede comet tail formation, and is later localized at the site of actin filament assembly between the polar bacterial surface and the front of the actin tail (Chakraborty *et al.*, *EMBO J.* 14:1314-1321, 1995). VASP is also a ligand for profilin (Reinhard *et al.*, *EMBO J.* 14:1583-1589, 1995). These findings suggest a role for VASP

in ordinary cytoskeletal dynamics, and as a host factor required for *Listeria* motility.

Although the recent discovery and preliminary characterization of Ena and VASP lends basic insight into cytoskeletal dynamics and bacterial motility, many questions remain concerning the specific mechanisms and regulation of these processes. Accordingly, there is a general need in the art for further discovery and characterization of structural and regulatory factors involved in microfilament dynamics affecting normal and abnormal cell morphology, adhesion, motility, growth and differentiation. Likewise, there remains a general need for additional discovery and characterization of factors involved in pathogenicity and motility of bacteria and other pathogens which rely on the induction of host cytoskeletal processes for transmission and virulence. Until these fundamental needs are satisfied, there will also remain more specific needs in the art for effective tools to model, diagnose and treat defects in cytoskeletal structure and regulation attributable to such diverse conditions as cancer, traumatic neural injury and cytopathologic bacterial infection.

SUMMARY OF THE INVENTION

It is therefore an object of the present invention to identify and characterize factors involved in microfilament dynamics affecting normal and abnormal cell morphology, adhesion, motility, growth and differentiation.

It is a further object of the invention to identify and characterize factors involved in microfilament dynamics affecting pathogenicity and motility of bacteria and other pathogens which rely on the induction of host cytoskeletal processes for transmission and virulence.

It is yet another object of the invention to provide tools to more effectively model, diagnose and treat defects in cytoskeletal structure and regulation attributable to injury and disease.

The invention achieves these objects and other objects and advantages which will become apparent from the description which follows by providing two novel mammalian

genes *Mammalian Ena (Mena)*, and *Ena-VASP like (Evl)* encoding novel proteins *Mena*, and *Evl*. The *Mena* and *Evl* proteins provided herein possess novel biochemical and cell biological properties rendering these proteins useful in the study and/or control of a variety of microfilament dynamic processes. In addition, the *Mena* and *Evl* proteins disclosed herein are useful for generating, isolating, and characterizing additional endogenous and exogenous factors, drugs and other agents useful for evaluating and/or controlling cytoskeletal dynamics involved in normal and abnormal cell morphology, adhesion, motility, growth and/or differentiation. Reflective of these uses, the invention provides several methods and tools specifically directed to the diagnosis and treatment of injury and disease conditions involving cytoskeletal dynamic processes, such as metastatic cancer and infection by cytopathogenic bacteria.

Within one aspect of the invention, purified and isolated polynucleotides are provided which encode a native *Mena* or *Evl* protein. Among these polynucleotides are DNA sequences encoding specific native isoforms of *Mena*, including an approximately 140 kD, neural-specific isoform of *Mena*, an approximately 60 kD hematopoietic-specific *Mena* isoform, and approximately 80 kD and 88 kD isoforms of *Mena* which are broadly distributed among different mammalian tissue types.

In related embodiments of the invention, *Mena* or *Evl* based polynucleotides are provided that encode *Mena* or *Evl* proteins or fragments thereof, recombinantly produced peptide derivatives, fusion proteins and the like incorporating only a portion of *Mena* or *Evl*, and up to the entire *Mena* or *Evl* protein. Among these *Mena* or *Evl* based polynucleotides are sequences that encode discrete functional domains of the *Mena* or *Evl* protein responsible for specific biological activities of the intact protein. Preferred functional domains in this context include an N-terminal domain of *Mena* and *Evl* that mediates binding of *Mena* to certain proline-rich ligands, including ActA, zyxin and vinculin, and which directs localization of *Mena* and heterologous proteins linked to the

EVH1 domain to focal contacts, and to the surface of cytopathogenic bacteria. Also provided are mutagenized polynucleotides that encode mutant analogs of the *Mena* or *Evl* protein, or of selected polypeptide portions of *Mena* or *Evl* such as the EVH1 functional domain, which exhibit modified biological activity compared to native *Mena* or *Evl* proteins and polypeptide domains.

Within another aspect of the invention, substantially pure *Mena* or *Evl* proteins are provided, including a variety of tissue specific or broadly distributed *Mena* and *Evl* isoforms disclosed herein. Also provided are *Mena* or *Evl* based synthetic peptides or fragments thereof, recombinantly produced peptides, fusion proteins and the like incorporating all or a portion of *Mena* or *Evl*, including peptides corresponding to the aforementioned *Mena* or *Evl* functional domains, as well as mutant analogs of the *Mena* or *Evl* protein or polypeptide portions thereof.

Within a related aspect of the invention, antibodies, antibody derivatives and chimeric antibodies are provided that bind to one or more of the *Mena* and *Evl* proteins and isoforms thereof. Also provided are antibodies, antibody derivatives and chimeric antibodies that bind to one or more of the *Mena* or *Evl* based synthetic peptides, recombinantly produced peptides, fusion proteins and the like disclosed herein.

Within yet another aspect of the invention, *Mena* or *Evl* based recombinant polynucleotide expression constructs are provided which include a polynucleotide insert encoding a *Mena* or *Evl* protein or *Mena* or Ena based synthetic peptide, recombinantly derived peptide, fusion protein or the like operably incorporated within an expression vector capable of directing expression of the polynucleotide insert sequence in a mammalian host cell. Various such constructs are provided, including constructs that incorporate polynucleotide sequences encoding the native 140 kD, 60 kD, 80 Kd or 88 Kd *Mena* isoforms. Also provided are related constructs that incorporate polynucleotide insert sequences encoding *Mena* or *Evl* proteins or fragments thereof, recombinantly derived

peptides, fusion proteins and the like including native or mutagenized forms of the *Mena* or *Evl* protein or of selected polypeptide portions of *Mena* or *Evl* such as the EVH1 functional domain.

Within related aspects of the invention, *Mena* or *Evl* based expression constructs are employed in methods for modulating expression of *Mena* or *Evl*, disrupting *Mena* or *Evl* expression or inducing ectopic expression of *Mena* or *Evl* genes and related polynucleotide sequences in mammalian host cells. The expression constructs are introduced into the host cells by microinjection, transfection or other suitable method to achieve transformation of the host cell, and the host cell is cultured, transplanted or left *in situ* under suitable conditions to allow the host cell to express the *Mena* or *Evl* based polynucleotide insert sequence.

Within additional aspects of the invention,a variety of screening and diagnostic methods and compositions are provided that employ one or more of the aforementioned inventive tools. For example, the invention provides screening, diagnostic and therapeutic methods that utilize *Mena* or *Evl* encoding polynucleotides, *Mena* or *Evl* proteins, peptides, fusion proteins and the like, *Mena* or *Evl* binding antibodies, *Mena* or *Evl* based expression constructs, and/or mammalian host cells transformed to express *Mena* or *Evl* insert sequences.

In preferred screening and diagnostic methods of the invention, labeled *Mena* or *Evl* proteins and peptides, as well as anti-*Mena* or anti-*Evl* antibodies, are used to determine the localization and activity of *Mena* or *Evl* in normal and abnormal cytoskeletal dynamic processes. In one such method, labeled *Mena* proteins, peptides and antibodies are introduced into the cytoplasm of *Listeria* infected cells to detect and quantify recruitment of *Mena* to the bacterial surface at the polar site of comet tail formation, and to detect and quantify the activity of *Mena* in mediating Listeria induction of microfilament polymerization. In more specific methods, labeled *Mena* and anti-*Mena* antibodies are used to detect and quantify *Mena* binding to ActA, profilin, zyxin or vinculin.

Each of these methods in turn provide a basis for additional methods to screen for agonists and antagonists of specific *Mena* activities involved in the *Listeria* microfilament induction pathway.

In related screening and diagnostic methods, *Mena* or *Evl* proteins and peptides, as well as anti-*Mena* or anti-*Evl* antibodies, are used to detect and quantify *Mena* or *Evl* activity in normal and abnormal cytoskeletal dynamic processes under endogenous control. As in the *Listeria* microfilament induction model, localization and quantification of *Mena* or *Evl* associated with dynamic cytoskeletal structures or events, or with specific molecular factors involved in cytoskeletal regulation, provides the basis for a variety of methods to diagnose abnormal cytoskeletal processes associated with disease, and to screen for agonists and antagonists of specific *Mena* activities involved in such processes. In one example, labeled *Mena* protein is used as a reagent to screen small molecule and peptide libraries to identify inhibitors of *Mena* activity. In more specific examples, an overlapping set of chemically synthesized peptides is generated covering the sequence of a selected *Mena* ligand, such as ActA, Profilin or Zyxin, and this library is screened using covalently or antibody labeled *Mena* to identify highly specific and potent peptide inhibitors.

In other related screening and diagnostic methods, *Mena* or *Evl* based synthetic peptides, recombinantly produced peptides, fusion proteins and the like incorporating all or a portion of *Mena* or *Evl*, as well as mutant analogs of *Mena* or *Evl* proteins or peptides, are used to screen for specific agonists and antagonists of *Mena* or *Evl* activities. In one such method, the EVH1 domain of *Mena* or *Evl*, or an EVH1 consensus peptide having a conserved amino acid sequence between *Mena*, *Evl* and VASP, is used as an affinity reagent to identify as yet unknown cellular factors involved in cytoskeletal regulation, or as a labeled probe to screen cDNA expression libraries to identify genes encoding such unknown regulatory factors.

Other screening and diagnostic methods of the invention rely on labeled polynucleotide probes to map the chromosomal locations of *Mena*, *Evl* and related genes, to identify genetic defects in these genes among cell populations or individuals, and to detect and measure expression of *Mena*, *Evl* or related genes in association with specific cytoskeletal dynamic structures, events or molecular regulatory factors.

In addition to screening and diagnostic methods, the invention provides a range of therapeutic methods for preventing and/or treating diseases and other conditions that involve aberrant cytoskeletal regulation, for example pathogenic infection, traumatic neural injury and cancer. Therapeutic methods of the invention variously utilize *Mena* or *Evl* encoding polynucleotides, *Mena* or *Evl* proteins and peptides, *Mena* or *Evl* binding antibodies, *Mena* or *Evl* based expression constructs, mammalian host cells transformed to express *Mena* or *Evl* insert sequences, and other therapeutic agents such as triplex forming oligonucleotides, antisense polynucleotides or ribozymes that specifically target *Mena* and/or *Evl* polynucleotides.

In preferred therapeutic methods directed to the treatment of pathogenic infection and cancer, it is useful to employ agents that inhibit *Mena* or *Evl* expression or activity and thereby eliminate or impair aberrant recruitment of *Mena* by pathogens, or interrupt *Mena* expression or activity associated with cancerous cell migration. Useful inhibitors in this context include antibodies that block or impair *Mena* or *Evl* by binding to the protein, *Mena* or *Evl* based, synthetic or recombinantly produced peptides, fusion proteins that compete for *Mena* or *Evl* binding partners or otherwise block or impair *Mena* or *Evl* expression or activity, triplex forming oligonucleotides, antisense polynucleotides or ribozymes that block expression of *Mena* and/or *Evl* polynucleotides, and mutant *Mena* or *Evl* based expression constructs that abolish or reduce *Mena* or *Evl* expression or activity in targeted cells.

In preferred therapeutic methods directed to the treatment of traumatic neural injury and other injury conditions where it is desirable to promote cell migration for

neural regeneration or wound healing, it is useful to employ agents that induce or enhance *Mena* or *Evl* expression or activity. Useful agents in this context include anti-*Evl* antibodies, *Mena* or *Evl* based, synthetic or recombinantly produced peptides, fusion proteins and the like that enhance *Mena* or *Evl* binding to its partners or otherwise enhance *Mena* or *Evl* expression or activity, and mutant *Metia or Evl* based expression constructs that induce or enhance *Mena* or *Evl* expression or activity in targeted cells.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides two novel mammalian genes *Mammalian Ena (Mena)*, and *Etia-VASP like (Evl)* which encode novel proteins *Mena* and *Evl*. Sequences encoding *Mena* and *Evl* include those sequences that result in minor variations in amino acid sequence, such as those due to genetic polymorphisms, allelic variations, differences between species and those in which blocks of amino acids have been added, altered or replaced without substantially altering the biological activity of the proteins. The *Mena* and *Evl* genes and *Mena* and *Evl* proteins disclosed herein are useful for generating, isolating, and characterizing endogenous and exogenous factors, drugs and other agents that can be employed in methods to evaluate and/or regulate cytoskeletal dynamic processes involved in normal and abnormal cell morphology, adhesion, motility, growth and/or differentiation. Within these general methods the invention provides specific methods directed toward the diagnosis and treatment of injury and disease conditions involving cytoskeletal dynamic processes, such as metastatic cancer and infection by cytopathogenic bacteria.

Within one aspect of the invention, purified and isolated polynucleotides are provided which encode a native *Mena* or *Evl* protein. Among these polynucleotides are DNA sequences encoding specific isoforms of native *Mena* or *Evl*. Preferred methods to isolate and purify polynucleotides encoding native *Mena* or *Evl* employ conventional screening, subcloning and polymerase chain reactions, for example

according to the methods and using the reagents described in Sambrook *et al.*, (1989) Molecular Cloning A Laboratory Manual (Cold Spring Harbor, NY: Cold Spring Harbor Press). In one example, a restriction fragment of the Drosophila gene *Ena* (Gertler *et al.*, *Genes Dev.* 9:521-533, 1995, which is incorporated herein by reference in its entirety) is used to probe a mammalian cDNA library made from mouse embryonic stems cells (Chen *et al.*, *Genes Dev.* 8:2293-2301, 1994, which is incorporated herein by reference in its entirety). Hybridization of the probe under stringent conditions is detected to identify positive phage candidates for incorporation of a *Mena* or *Evl* polynucleotide. Briefly, the positive phage are then purified and their cDNA inserts are subcloned and sequenced. Additional polynucleotide probes that are useful within the invention include *Mena* or *Evl* probes corresponding to partial or complete nucleotide sequences generated from the cDNAs depicted in SEQ ID. NOS. 1, 3 or 7 below. Degenerate oligonucleotides based on the amino acid sequences of SEQ ID NOS. 2, 4, 5, 6, 8 and 9 are also useful in methods for identifying additional isoforms and related sequences. Alternately, as set forth in more detail below, proteins, synthetic peptides, recombinantly produced peptides, fusion proteins and the like that bind or otherwise interact with *Mena* can be used as labeled probes to screen mammalian cDNA expression libraries to identify additional genes or cDNAs encoding *Mena* or *Evl* proteins. In one such embodiment, known *Mena* binding partners including ActA, zyxin, vinculin and VASP, are used as probes to detect *Mena* encoding polynucleotides, for example using well known autoradiographic or immunoassay screening methods.

The choice of hybridization conditions will be evident to one skilled in the art and will generally be guided by the purpose of the hybridization, the type of hybridization (DNADNA or DNA-RNA), and the level of relatedness between the sequences. Methods for hybridization are well established in the literature; see, for example: Sambrook, *ibid.*; Hames and Higgins, eds, *Nucleic Acid Hybridization, A Practical Approach*, IRL Press, Washington DC, 1985; Berger and Kimmel,

eds, *Methods in Enzymology*, Vol. 52, *Guide to Molecular Cloning Techniques*, Academic Press Inc., New York, NY, 1987; and Bothwell, Yancopoulos and Alt, eds, Methods for *Cloning and Analysis of Eukaryotic Genes*, Jones and Bartlett Publishers, Boston, MA 1990; which are incorporated by reference herein in their entirety. One of ordinary skill in the art realizes that the stability of nucleic acid duplexes will decrease with an increased number and location of mismatched bases; thus, the stringency of hybridization may be used to maximize or minimize the stability of such duplexes. Hybridization stringency can be altered by adjusting the temperature of hybridization; adjusting the percentage of helix-destabilizing agents, such as formamide, in the hybridization mix; and adjusting the temperature and salt concentration of the wash solutions. In general, the stringency of hybridization is adjusted during the post-hybridization washes by varying the salt concentration and/or the temperature. Stringency of hybridization may be reduced by reducing the percentage of formamide in the hybridization solution or by decreasing the temperature of the wash solution. High stringency conditions may involve high temperature hybridization (e.g., 65-68°C in aqueous solution containing 4-6XSSC, or 42°C in 50% formamide) combined with high temperature (e.g., 5-25°C below the T_m) and a low salt concentration (e.g., 0. 1X SSC). Reduced stringency conditions may involve lower hybridization temperatures (e.g., 35-42°C in 20-50% formamide) with intermediate temperature (e.g., 40-60°C) and washes in a higher salt concentration (e.g., 2-6X SSC). Moderate stringency conditions, which may involve hybridization at a temperature between 50°C and 55°C and washes in 0.1x SSC, 0.1% SDS at between 50°C and 55°C, may be used to identify clones encoding polynucleotide molecules encoding *Mena* or *Evl* from other species or to isolate isoforms of *Mena* or *Evl*.

Mena or *Ena* encoding polynucleotides that are particularly useful within the invention encode *Mena* or *Ena* proteins having unique structural and/or biochemical properties, or which exhibit discrete expression or tissue

distribution patterns among various *Mena* or *Ena* isoforms. Within the specific examples identified, SEQ ID NO. 1 depicts a *Mena* cDNA polynucleotide and is deduced amino acid sequence (SEQ ID NOS. 1 and 2) evidently expressed in two isoforms, approximately 80 kD, and 88 kD in size, and exhibiting a broad pattern of tissue distribution in neurons, fibroblasts, kidney epithelium, muscle, neural crest and hematopoietic cells. Other examples identify *"Neural Mena"* cDNA polynucleotides that contain combinations of three alternately included exons and encoding multiple isoforms of *Mena* (Neural *Mena*$^{+}$, SEQ ID NO. 4; Neural *Mena*$^{++}$, SEQ ID NO. 5; and Neural *Mena*$^{+++}$, SEQ ID NO. 6). In a representative example, a cDNA encoding Neural *Mena* is disclosed (SEQ ID NO. 3). Unlike *Mena*, Neural *Mena* isoforms are approximately 140 kD in size and exhibit neural tissue specific distribution. In yet other examples, cDNA polynucleotides encoding an approximately 60 kD, hematopoietic specific form of *Mena*, and cDNA polynucleotides (SEQ ID NOS. 7) encoding the *Evl* protein (SEQ ID NO. 8) are described.

To evaluate size and distribution of *Mena* or *Evl* proteins encoded by different polynucleotides, a variety of well known methods can be used. For example, molecular size of proteins translated from *Mena* or *Ena* coding cDNAs *in vitro* can be determined by gel electrophoresis, and tissue distribution can be determined by comparative Western blotting of cell lysates from different tissues, or *in situ* hybridization of whole embryos or tissue sections, using anti-*Mena* or anti-*Evl* antibodies (for example according to the general methods disclosed in Harlow and Lane (1988); *Antibodies A Laboratory Manual* (Cold Spring Harbor, NY: Cold Spring Harbor Press), which is incorporated herein by reference in its entirety). Other methods of determining the tissue specificity of *Mena* or *Ena* encoding polynucleotides include assays that measure *Mena* or *Ena* expression between tissue differentiation stages, for example in *in vivo* developmental models or systems where tissue differentiation can be induced by exogenous stimuli. In one such example disclosed herein, *Mena* protein is assayed by direct anti-*Mena* immunofluorescence assay in developing embryos and in

differentiated P19 embryonic carcinomal cells, to demonstrate that expression of the 140 kD neural specific *Mena* isoform increases during development at the time of rapid neurite outgrowth, and that subcellular distribution of Neural *Mena* in P19 cells is concentrated in the growth cones of developing neurites. In a related assay, anti-*Mena* Western blots of lysates from P19 embryonic carcinomal cells following retinoic acid induction of the cells to differentiate neurofilament-positive neurons (Rudnicki and McBurney, (1987) In *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E. Robertson, ed., (Oxford, England: IRL Press Limited), pp. 19-47; which is incorporated herein by reference in its entirety) demonstrated that the 140 kD Neural *Mena* isoform is induced during the neural differentiation process.

Also of particular use within the invention are *Mena* or *Evl* based polynucleotides that encode *Mena* or *Evl* proteins or fragments thereof, recombinantly produced peptide derivatives, fusion proteins and the like incorporating only a portion of *Mena* or *Evl*, and up to the entire *Mena* or *Evl* protein. Among these *Mena* or *Evl* based polynucleotides are sequences that encode discrete functional domains of the *Mena* or *Evl* protein responsible for specific biological activities of the intact protein. Functional domains which are of particular interest in this context will be directly involved in structural or regulatory interactions mediating cytoskeletal dynamic processes, for example localization of *Mena* or *Evl* to structures involved in cytoskeletal dynamic processes, or functional association of *Mena* or *Evl* with molecular factors that mediate or regulate cytoskeletal dynamic processes. In one example, an N-terminal domain of *Mena* (N-*Mena*) including approximately the first 170 amino acids of the protein is demonstrated to mediate binding of *Mena* to certain proline-rich ligands, including ActA, zyxin and vinculin, and to direct localization of *Mena* and heterologous proteins linked to the N-*Mena* peptide to focal contacts, and to the surface of cytopathogenic bacteria. The N-*Mena* peptide corresponds to a functional domain (the EVH1 domain) that is highly conserved between *Mena*, *Evl* and VASP,

as demonstrated by sequence alignment comparisons. A consensus sequence for the alignment is shown in SEQ ID NO. 9.

Within a closely related aspect of the invention, substantially pure *Mena* or *Evl* proteins and peptides are provided that are initially encoded by the aforementioned polynucleotides. The *Mena* and *Evl* proteins include a variety of tissue specific or broadly distributed *Mena* and *Evl* isoforms disclosed herein. Also provided are *Mena* or *Evl* based synthetic peptides, recombinantly produced peptides, fusion proteins and the like incorporating all or a portion of *Mena* or *Evl*, including peptides corresponding to the aforementioned *Mena* or *Evl* functional domains, as well as mutant analogs of the *Mena* or *Evl* protein or polypeptide portions thereof. The proteins or polypeptide portions thereof may or may not have the biological activity of corresponding native *Mena* or *Evl*. Within one example, proteins are prepared that are capable of binding to the proline-rich binding partner ActA, but not capable of inducing profilin.

The peptides can be prepared via chemical synthesis, as described hereinbelow, or by recombinant DNA technology, or as fusion proteins, and the like. Desirably, the peptide will be as small as possible while still maintaining substantially all of the reactivity of a larger peptide. The subject peptides have an amino acid sequence encoded by a nucleic acid which hybridizes under stringent conditions with an oligonucleotide of 15 or more contiguous nucleotides of SEQ ID NOS. 1, 3, or 7. Exemplary amino acid sequences among the subject peptides are shown in SEQ ID NO. 2 (amino acids 6-170 (N-Mena); amino acids 440-537 (C-Mena) and SEQ ID NO. 11. It will be understood that the peptides of the present invention or analogs thereof which have *Mena* or *Evl* activity may be modified from a native *Mena* or *Evl* sequence as necessary to provide other desired attributes, e.g., improved binding or inhibitory activity (e.g., increased competition with native protein), improved adsorption to a solid phase, etc. For instance, the peptides may be subject to various changes, such as insertions, deletions, and substitutions, either

conservative or nonconservative, where such changes might provide for certain advantages in their use. By conservative substitutions is meant replacing an amino acid residue with another which is biologically and/or chemically similar, e.g., one hydrophobic residue for another, or one polar residue for another. The substitutions include combinations such as Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln; Ser, Thr; Lys, Arg; and Phe, Tyr. Usually, the sequence of the peptide will not differ by more than about 20% from the native *Mena* or *Evl* sequence, except where additional amino acids may be added at either terminus for the purpose of modifying the physical or chemical properties of the peptide for, e.g., ease of linking or coupling, and the like. Having identified different peptides of the invention, in some instances it may be desirable to join two or more peptides in a composition or admixture. The peptides in the composition can be identical or different, and together they should provide equivalent or greater reactivity than the parent peptide(s). The subject peptides find a variety of uses including preparation of specific antibodies. Because of their relatively short size, the peptides can be synthesized in solution or on a solid support in accordance with conventional techniques. Various automatic synthesizers are commercially available and can be used in accordance with known protocols. See, for example, Stewart and Young, *Solid Phase Peptide Synthesis*, 2d. ed., Pierce Chemical Co. (1984); Tam *et al.*, *J. Am. Chem. Soc.* 105:6442 (1983); Merrifield, *Science* 232:341-347 (1986); and Barany and Merrifield, *The Peptides*, Gross and Meienhofer, eds., Academic Press, New York, pp. 1-284 (1979), each of which is incorporated herein by reference. Short peptide sequences, or libraries of overlapping peptides, usually from about 6 up to about 35 to 50 amino acids, which correspond to the selected regions described herein, can be readily synthesized and then screened in screening assays designed to identify reactive peptides. Alternatively, recombinant DNA technology may be employed wherein a nucleotide sequence which encodes a peptide of the invention is inserted into an expression vector, transformed or transfected into an

appropriate host cell and cultivated under conditions suitable for expression. These procedures are generally known in the art, as described generally in Sambrook *et al.*, *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring Harbor, New York (1989); Ausubel *et al.*, (ed.) *Current Protocols in Molecular Biology*, John Wiley and Sons, Inc., New York (1987), and U.S. Pat. Nos. 4,237,224, 4,273,875, 4,431,739, 4,363,877 and 4,428,941, for example, whose disclosures are incorporated herein by reference.

The various biological activities of EVH1 and other functional domains of *Mena* or *Evl* can be detected and quantified using a variety of methods, including for example ligand binding assays using immobilized *Mena* or *Evl* proteins or fusion proteins. In one such method, PCR is used to generate polynucleotide fragments encoding amino acids corresponding to a selected or putative functional domain, for example amino acids 6-170 representing N-Mena, or amino acids 440-537 representing a C-terminal functional domain of *Mena* (C-Mena). The polynucleotide fragments are then cloned into a suitable expression vector, for example pGex2T, and fusion proteins are prepared (Smith and Johnson, *Gene* 67:31-40, 1988, which is incorporated herein by reference in its entirety). The fusion proteins can be endogenously labeled, for example using a covalent radiolabel or other conventional marker, or alternately labeled using antibodies that bind to the fusion protein. In one ligand binding example disclosed herein, an N-Mena-Gst fusion protein was generated and immobilized on glutathione-agarose bead matrix (SEPHAROSE; Pharmacia Biotech, Inc., Piscataway, NJ). Proteins capable of binding N-Mena-Gst were then purified from head lysates of stage E12 mouse embryos, or human erythrocytic leukemia cells (HEL). Bound proteins were analyzed by Western blotting with antibodies to ActA, zyxin, vinculin or the Ack kinase. These and other exemplary studies employing appropriate Gst binding controls demonstrate that N-Mena binds to ActA, zyxin and vinculin, but not to Ack. More specific examples, employing ligand overlay assays using peptides derived from vinculin or ActA (for example according to the methods of Chakraborty *et al.* *EMBO J.*

14:1314-1321, 1995) demonstrate that N-Mena can associate with motifs common to ActA, zyxin and vinculin.

The activity of N-Mena and other functional domains of *Mena* and/or *Evl* in cytoskeletal dynamic processes can be further evaluated and incorporated within the methods of the invention using a variety of additional tools that are disclosed herein, or are elsewhere described and known in the art. In one example disclosed herein, purified Gst-N-Mena is microinjected into PtK2 cells seeded to semi-confluency on CELLocate coverslips (Eppendorf, Hamburg, Germany), and anti-Gst antibody assays coupled with phalloidin labeling to show the distribution of microfilaments is used to demonstrate that the injected fusion protein localizes to focal adhesions, whereas Gst injected alone is diffusely localized throughout the cytoplasm. Subsequently a peptide containing the ActA sequence SEPSSFEFPPPPTDEELRLA (SEQ ID NO. 12) is injected into the cells in an attempt to saturate the N-Mena binding site for ActA. Following the peptide injection, Gst-N-Mena is observed to be depleted from the focal contacts, whereas distribution of the fusion protein is unaffected by injection of an unrelated ActA peptide. Similar competition assays demonstrate depletion of endogenous *Mena* in ActA peptide injected cells. These examples and other examples provided herein establish that the EVH1 functional domain of *Mena* is necessary and sufficient for directing *Mena* and N-Mena based fusion proteins to focal adhesions and other sites associated with cytoskeletal dynamic processes via direct protein:protein interactions with zyxin, vinculin or other focal adhesion proteins containing an ActA-like motif.

The activity of *Mena* or *Evl* and N-Mena and other functional domains of *Mena* and/or *Evl* in cytoskeletal dynamic processes can be further evaluated and incorporated within the methods of the invention with the aid of protein crystallography. The basic protein expression methods of the invention provide for the production of sufficiently large amounts of pure *Mena* and *Evl* protein to allow crystallographic analysis of *Mena* and *Evl* bound and unbound to their various binding partners. These studies will further elucidate the

complex structure and function of the *Mena* and *Evl* proteins and will be of substantial value in the design of small molecule inhibitors of these proteins.

The activity of N-Mena and other functional domains of *Mena* and/or *Evl* in cytoskeletal dynamic processes can be further evaluated and incorporated within the methods of the invention in the context of pathogenic induction of cytoskeletal dynamic processes. In one example disclosed herein involving microfilament induction by the cytopathogenic bacterium *Listeria*, purified Gst-N-Mena was microinjected into PtK_2 cells which were then infected with *Listeria*. Anti-Gst immunoassay coupled with phalloidin labeling shows that the *Mena* based fusion protein associates with the surface of the bacteria and overlaps with actin in the region of actin polymerization at the tail of the bacterium, similar to the distribution of endogenous *Mena* in *Listeria* infected cells. Further exemplary studies show that *Mena* protein translated *in vitro* binds immobilized profilin, which binding is reduced by six-fold following preincubation of the profilin matrix with a $(GP_5)_3$ peptide (SEQ ID NO. 12) corresponding to the profilin-binding motif of VASP (Reinhard *et al.*, *EMBO J.* 14:1583-1589, 1995, which is incorporated herein by reference in its entirety). These examples and other examples provided herein establish that the EVH1 functional domain of *Mena* is necessary and sufficient for directing *Mena* and N-Mena based fusion proteins to the surface of *Listeria* at the region of actin polymerization to form the bacterial comet tail. Further, these examples establish that *Mena* contains two distinct domains capable of mediating interactions with ActA, or profilin in association with cytoskeletal dynamic processes required for *Listeria* pathogenicity.

The general methods and compositions described above for localizing and quantifying *Mena* or *Evl* associated with dynamic cytoskeletal structures or events, and for determining *Mena* activity in association with specific factors and events involved in cytoskeletal regulation, provides the basis for a variety of methods to diagnose and treat abnormal cytoskeletal processes associated with pathogenic infection, disease and

other conditions, and to screen for agonists and antagonists of specific *Mena* activities involved in such processes. A particularly us ful set of tools in this context includes the various antibodies provided within the invention for detecting *Mena* and/or *Evl* expression, activity and localization. Antibody reagents useful for these purposes include antibodies, antibody derivatives and chimeric antibodies that bind to one or more of the *Mena* and *Evl* proteins and isoforms thereof. Also provided are antibodies, antibody derivatives and chimeric antibodies that bind to one or more of the *Mena* or *Evl* based synthetic peptides, recombinantly produced peptides, fusion proteins and the like disclosed herein.

General methods for the production of non-human antisera or monoclonal antibodies (e.g., murine, lagomorpha, porcine, equine) are well known and may be accomplished by, for example, immunizing an animal with *Mena* protein or *Mena* based peptides or alternatively with *Evl* protein or *Evl* based peptides. Within one example, for the production of monoclonal antibodies to *Mena*, antibody producing cells are obtained from immunized animals, immortalized and screened, or screened first for the production of an antibody that binds to the *Mena* protein or peptides and then immortalized. It may be desirable to transfer the antigen binding regions (i.e., F(ab')2 or hypervariable regions) of non-human antibodies into the framework of a human antibody by recombinant DNA techniques to produce a substantially human molecule. Methods for producing such "humanized" molecules are generally well known and described in, for example, U.S. Patent No. 4,816,397, which is incorporated by reference herein in its entirety.

Alternatively, a human monoclonal antibody or portions thereof may be identified by first screening a human B-cell cDNA library for DNA molecules that encode antibodies that specifically bind to *Mena* according to the method generally set forth by Huse *et al.* (*Science* 246:1275-1281, 1989, which is incorporated by reference herein in its entirety). The DNA molecule may then be cloned and amplified

to obtain sequences that encode the antibody (or binding domain) of the desired specificity.

Anti-Mena or anti-*Evl* antibodies are particularly useful within the invention as labeled reagents to detect, image and/or quantify the presence or activity of *Mena* or *Evl*, or of agonists or antagonists of *Mena* or *Evl* activity. In this context, anti-Mena and anti-*Evl* antibodies are provided which incorporate one or more well known labels such as a dye, fluorescent tag or radiolabel. Anti-Mena or anti-*Evl* antibodies may also be used themselves as agonists or antagonists of *Mena* or *Evl* activity, for example by interfering with the binding of *Mena* to ActA, profilin, zyxin or vinculin. In addition, anti-Mena or anti-*Evl* antibodies may be used as targeting agents for the delivery of compounds of therapeutic interest. Such compounds include, but are not limited to, toxins, cytostatic compounds, or proenzymes whose potential function is to activate endogenous proenzymes, to activate proenzymes from exogenous sources, or to activate enzyme cleavage sites on prodrugs. Also contemplated within the invention are bifunctional antibodies having independent antigen binding sites on each immunoglobulin molecule (as disclosed for example in *Thromb. Res. Suppl.* X:83, 1990, and in *The Second Annual IBC International Conference on Antibody Engineering*, A. George ed., Dec. 16-18, 1991), as well as panels of antibodies having differing specificities. Bifunctional antibodies and antibody panels of particular use within the invention include antibodies and panels of antibodies that bind to both *Mena* and *Evl*, or to multiple functional domains of the *Mena* or *Evl* protein.

The subject proteins and peptides of the invention are also useful as reagents to detect, image and/or quantify the presence or activity of *Mena* or *Evl*, or of agonists or antagonists of *Mena* or *Evl* activity, in addition to their usefulness in the preparation of anti-*Mena* and anti-*Evl* antibodies. In this context a collection of *Mena* or *Evl* proteins substantially purified are provided, including all of the *Mena* and *Evl* isoforms disclosed herein. The *Mena* and *Evl* proteins produced according to the present invention may be

purified using a number of established methods such as affinity chromatography using anti-*Mena* or anti-*Evl* antibodies coupled to a solid support. Additional purification may be achieved using conventional purification means such as liquid chromatography, gradient centrifugation and gel electrophoresis among others. Methods of protein purification are known in the art (see generally, Scopes, R., *Protein Purification*, Springer-Verlag, NY, 1982, which is incorporated herein by reference) and may be applied to the purification of recombinant *Mena* or *Evl* described herein. Thus, *Mena* and *Evl* are provided isolated from its natural cellular environment, and substantially free of other cellular proteins. Purified *Mena* and *Evl* are provided, where substantially pure *Mena* and *Evl* of at least about 50% is preferred, at least about 70-80% is more preferred, and 95-99% or more homogeneity most preferred. Once purified, partially or to homogeneity, as desired, the recombinant *Mena* and *Evl* or native *Mena* and *Evl* may be used to generate antibodies, diagnostically in assay procedures, etc.

Also provided are *Mena* or *Evl* based synthetic peptides, recombinantly produced peptides, fusion proteins and the like incorporating all or a portion of *Mena* or *Evl*. Proteins, peptides and fusion proteins of the invention may be selected to include one or more of the *Mena* or *Evl* functional domains disclosed herein, as well as mutant analogs of the *Mena* or *Evl* protein or polypeptide which exhibit modified biological activity compared to native *Mena* or *Evl* proteins and polypeptide domains.

The proteins, peptides and fusion proteins of the invention are provided according to the methods disclosed herein, as well as by additional recombinant, biosynthetic and peptide chemical methods described elsewhere and generally known in the art. Of particular interest among these methods are site directed mutagenic techniques that can be used to generate targeted mutations in specific regions or functional domains of a *Mena* or *Evl* based polynucleotide. For example, it will be generally appreciated that site directed mutagenesis within the EVH1 domain encoding portion of *Mena*

will yield *Mena* mutants that are either hyperfunctional or dominant negative with respect to *Mena* activities attributable to the EVH1 domain, including ActA, zyxin and vinculin binding activities, and subcellular localization of *Mena*. Such mutants can be useful by themselves in various applications, or combined with other native or mutant peptide sequences, labels or other agents for more specific purposes. For example, an N-Mena mutant having an enhanced ActA, zyxin or vinculin binding activity selective for a specific binding partner can be used to screen for particularly strong or specific antagonists that inhibit that specific binding activity. Likewise, hyperfunctional *N-Mena* mutants will be particularly useful as gene therapeutic agent to induce or enhance *Mena* function in impaired systems, such as in the case of a neural injury or wound healing condition. In more detailed examples, hyperfunctional N-Mena mutants can be operably joined to other peptide sequences, labels or chemical agents to achieve a particular therapeutic value. One such chimeric mutant is contemplated having a hyperfunctional N-Mena mutation as well as a dominant negative mutation in the proline rich, profilin binding domain. This particular chimera will compete strongly with endogenous *Mena* for ActA, zyxin and vinculin binding, but will fail to promote wild type profilin binding and microfilament assembly. Such a mutant will be useful tool in a variety of applications, for example to screen for agonists that replace or enhance *Mena*-profilin binding activity, or as a gene therapeutic agent capable of inhibiting endogenous *Mena* function in aberrant migratory cells, such as Chronic Myelogenous Leukemia (CML) and other cancer cells.

In further aspects of the invention, *Mena* or *Evl* proteins, peptides, fusion proteins and antibodies are used in a variety of screening and diagnostic methods. As will be evident to the common practitioner, the polynucleotide molecules, protein, peptides and antibodies of the present invention are useful in *in vitro* assays to screen for compounds capable of modulating the activity of expression of *Mena* or *Evl*. Within such assays, test compounds may be

assessed for their ability to increase or decrease *Mena* activity or expression relative to a control assay in which the test compound is absent. Screening assays of the present invention exploit the binding of *Mena* and *Evl* to different substrates. Within one embodiment, test compounds are screened for the ability to compete with *Mena* binding partners such as ActA, profilin, Zyxin or Vinculin. Within another embodiment, test compounds are screened for the ability to modulate *Mena* or *Evl* activity by increasing or decreasing *Mena* or *Evl* expression.

In preferred diagnostic methods, labeled *Mena* or *Evl* proteins, peptides, or anti-*Mena* or anti-*Evl* antibodies are employed to detect expression, localization and/or activity of *Mena* or *Evl* associated with normal and/or abnormal cytoskeletal structures or processes, or in association with specific molecular factors involved in cytoskeletal regulation. In one general diagnostic example, *Mena* or *Evl* expression or activity is detected and/or quantified in a normal cell population or tissue, and these results are compared to *Mena* or *Evl* expression or activity detected and/or quantified in a test cell population or tissue (for example a population of cancerous cells or cells from a site of neural injury). Detection and/or quantification of *Mena* or *Evl* expression, localization or activity can be accomplished by a variety of methods, such as by in situ hybridization using anti-*Mena* or anti-*Evl* antibodies on embryos or tissue sections or within antibody microinjected cells, by Western blotting or immunoprecipitation using anti-*Mena* or anti-*Evl* antibodies in cell or tissue lysates, or by affinity purification using anti-*Mena* or anti-*Evl* antibodies bound to a solid phase, among other methods. Comparable methods are disclosed herein, or are elsewhere disclosed and known in the art, for using non-antibody agents to detect and/or quantify *Mena* or *Evl* expression or activity. Suitable non-antibody probes for use within these methods include for example oligonucleotide probes that hybridize to *Mena* or *Evl* transcripts, labeled binding partners of *Mena* or *Evl* such as ActA, zyxin, vinculin or profilin, and synthetic or

recombinant peptide analogs of *Mena* or *Evl* binding partners, among other useful probe types. For example, *Mena* and *Evl* cDNA and oligonucleotide probes may be useful in Northern, Southern, and dot-blot assays for identifying and quantifying the level of expression of *Mena* or *Evl* in a cell. Measuring the level of *Mena* or *Evl* expression may provide prognostic markers for assessing the growth rate and invasiveness of tumors. In addition, considering the role of *Mena* in axonogenesis, birth defects and abortions may result from the absence or expression of an abnormal *Mena* protein. In this case, *Mena* may be useful in prenatal screening of mothers and/or for *in utero* testing of fetuses.

Differences that are detected and/or quantified between *Mena* or *Evl* expression or activity between normal and test cell populations or tissues may be diagnostic of particular disease states or other conditions characterized by aberrant cytoskeletal structure or regulation. In the case of cancerous or precancerous test cells, such as CML cells, an increase of *Mena* expression compared to control cells is predictive of an increased risk of metastatic disease due to *Mena*-mediated cell motility and reduction of contact inhibition. In the case of test cells taken from sites of neural injury, the level of *Mena* expression or activity compared to control cells is predictive of the extent of neural regeneration that can be expected in a particular case, and may also be useful for determining preferred courses of treatment. In the case of test cells suspected of carrying *Listeria* infection, or infection by any of a number of other pathogenic agents that induce cytoskeletal dynamic processes in infected cells, the presence and pattern of *Mena* expression in association with the pathogen provides a positive diagnosis of the type of infection, and the level of *Mena* expression or activity compared to control cells is diagnostic of the severity of infection.

Additional diagnostic methods of the invention rely on labeled polynucleotide probes to map the chromosomal locations of *Mena*, *Evl* and related genes, to determine linkage of these genes relative to other genes, and to identify

genetic defects in these genes among cell populations or individuals. To facilitate these methods, *Mena* was demonstrated by genetic linkage analysis to be located in the mouse to chromosome 1a offset 73 in the Jackson backcross hybrid mapping panel. This location in the mouse is syntenic to a human chromosomal position of 1Q21-23 (Stanier *et al.*, *Genomics* 26:473-478, 1995), which location adds significance to the methods of the invention because it is a putative hot spot for breast cancer.

Yet additional diagnostic methods of the invention rely on the detection and/or quantification of phosphorylation of *Mena* or *Evl*. Cytoskeletal dynamic processes are mediated by a variety of signal cascades that can be induced by external stimuli such as adhesion through integrins or stimulation with a growth factor such as PDGF. Many of these signals are propagated in part by kinase cascades that may trigger *Mena* phosphorylation. In this context, it is noteworthy that Src family kinases are concentrated in neuronal growth cones (Bixby and Jhabvala, *J. Neurosci.* 13:3421-32, 1993), and c-Src may be required for proper neurite outgrowth on the cell adhesion molecule L1 (Ignelzi *et al.*, *Neuron* 12:873-84, 1994). Mammalian c-Abl is a another logical candidate *Mena* kinase. From studies in NIH3T3 cells, c-Abl is widely perceived as a largely nuclear tyrosine kinase. However, in P19 neurons, Abl is found exclusively in the cytoplasm and in growth cones. A role for cytoplasmic c-Abl in actin dynamics is indicated by the presence of G- and F-actin binding activities in its C-terminus (McWhirter *et al.*, *Mol. Cell. Biol.* 13:7587-95, 1993; McWhirter *et al.*, *Mol. Cell. Biol.* 11, 1553-1565, 1991; McWhirter and Wang, *EMBO J.* 12:1533-1546, 1993; Van Etten *et al.*, *J. Cell. Biol.* 124:325-340 1994). Evaluating the potential involvement of *Mena* in normal c-Abl function, and in neoplasias induced by Abl oncogenes, is an important object of the invention. In this context, it is shown herein that *Mena* contains serine residues aligning to known cyclic nucleotide kinase-dependent phosphorylation sites in VASP (Butt *et al.*, *J. Biol. Chem.* 269:14509-17, 1994). These serine residues in *Mena* flank the

central proline-rich region of the protein. *Evl* contains the amino-terminal site only, while *Ena* lacks both sites. In the examples below it is also shown that a slower migrating form of *Mena* is less efficiently recovered in SH3 binding assays than a faster form. These and other data suggest that some interactions mediated by the proline-rich core of *Mena* may be regulated by post-transitional modification of the protein, such as serine-threonine phosphorylation. Tyrosine phosphorylation of the larger, neural isoform of *Mena* may promote interactions with molecules that contain SH2 or PTB domains and link *Mena* to other signaling complexes. In addition, the alternatively-included portions of the 140 kD *Mena* isoform may contain the phosphorylated tyrosine(s), or sequences that recruit *Mena* kinases. These findings indicate that diagnostic and screening methods of the invention will be useful to identify and characterize any cytoskeletal dynamic processes involving *Mena* or *Ena* phosphorylation, to diagnose defects in such processes, and to screen for modulators of such processes that may be useful in therapeutic and other applications.

The same steps and compositions that are employed within diagnostic methods of the invention are readily adapted for use within powerful screening methods provided by the invention. Screening methods that are particularly useful within the invention include high throughput screening assays designed to identify modulators of *Mena* or *Evl* expression or activity. In preferred screening assays, labeled *Mena* or *Evl* proteins, peptides, or anti-Mena or anti-*Evl* antibodies are employed in a similar manner as described above to detect and/or quantify expression or activity of *Mena* or *Evl* in comparable test and control samples. Useful control samples in this context generally include a variety of *in vivo* or *in vitro* assay mixtures suitable for detecting and/or quantifying *Mena* or *Evl* binding to a selected binding partner, for example ActA, zyxin, vinculin or profilin. Other suitable control samples include *in vivo* or *in vivo* assays mixtures suitable for detecting other activities of *Mena* or *Evl*, for example subcellular localization of *Mena* to the surface of a

cytopathogenic bacteria or a site of endogenously controlled microfilament formation, or *Mena* or *Evl* phosphorylation. Useful test samples within these screening methods contain an added test substance, i.e. a putative *Mena* or *Evl* modulating agent, in qualitatively or quantitatively comparable assay mixtures to those of the control samples. In screens aimed at detecting modulators of *Mena* or *Evl* binding to a selected binding partner, the test sample contains suitable amounts of *Mena* or *Evl* protein and a selected binding partner under conditions that permit the formation of Mena- or *Evl*-binding partner complexes in the absence of the test substance. The complexes are then detected and/or quantified according the methods disclosed herein, and these results are compared to the results of detection and/or quantification of *Mena*- or *Evl*-binding partner complexes formed in the control sample.

Also provided are kits and multicontainer units comprising reagents and components for practicing the assay methods of the present invention. Kits of the present invention may, in addition to reagents for detecting *Mena* and *Evl*, contain enzymatic reagents such as reverse transcriptase or polymerase; suitable buffers; nucleoside triphosphates; suitable labels for labeling the reagents for detecting *Mena* and *Evl* and developing reagents for detecting the signal from the label. In one aspect, kits of the present invention contain sequence-specific oligonucleotide primers for detecting polynucleotide molecules encoding *Mena* and *Evl*. Such primers may be provided in separate containers or may be provided in combinations of one or more primer pairs in a series of containers. One aspect of the invention provides kits containing *Mena* and *Evl* sequence-specific probes. Within yet another aspect, kits contain antibodies useful for detecting *Mena* and *Evl* (or mutants thereof) in a sample. Such kits contain *Mena*- and/or *Evl*-specific antibodies for detecting *Mena* and *Evl* protein. The *Mena* and *Evl*-specific antibodies may be labeled or may be detected by binding to a secondary antibody. The antibody reagents may be provided in separate container or may be provided in combination in a series of containers. In addition to these components, the

kits may also contain instructions for carrying out the assay and/or additional containers suitable for carrying out the reactions of the assay.

The complex structure of the *Mena* and *Evl* proteins, and the various biological activities of EVH1 and other functional domains of *Mena* or *Evl*, provide for a broad array of potential screening assays and assay formats. Among these various screening methods ligand binding assays using immobilized *Mena* or *Evl* proteins or fusion proteins are preferred for high throughput screening purposes. In one such method, labeled *Mena* or *Evl* proteins or *Mena* or *Evl* based peptides or fusion proteins are immobilized on a solid phase, for example, an N-Mena-Gst fusion protein immobilized on a glutathione-agarose bead matrix such as glutathione SEPHAROSE (Pharmacia Biotech, Inc., Piscataway, NJ). The control assay sample is completed by exposing the bound N-Mena-Gst fusion protein to a N-*Mena* ligand, for example ActA, zyxin or vinculin. The ligand may be provided in a purified form, or as a component of a ligand-containing mixture, for example a cell or tissue lysate. N-Mena-ligand complexes are detected and/or quantified following exposure of the ligand to the bound fusion protein using any of a variety of detection or quantification methods disclosed herein, for example by a selected immunoassay using antibodies to ActA, zyxin or vinculin. The test assay sample is prepared in a comparable fashion, by exposing the bound N-Mena-Gst fusion protein to an N-Mena ligand under conditions that permit formation of N-Mena-ligand complexes in the control assay sample. However, prior to detection and/or quantification of N-Mena-ligand complexes in the control assay sample, a test substance is included therein. Generally the test substances is added in the form of a purified agent, however it is also contemplated that test substances useful within the invention may include substances present throughout the handling of test sample components, for example host cell factors that are present in a cell lysate used for generating the test sample. Such endogenous factors may be segregated between the test and control samples for example by using different cell types for

preparing lysates, where the cell type used for preparing the test sample expresses a putative test substance that is not expressed by the cell type used in preparing the control sample. A particularly useful set of test and control cell types in this context are P19 embryonic carcinomal cells that have and have not been induced to undergo neural differentiation by retinoic acid.

More specific screening methods of the invention are also disclosed herein, including ligand overlay assays using peptides derived from *Mena* or *Evl* binding partners as test substances (for example according to the methods of Chakraborty *et al. EMBO J. 14*:1314-1321, 1995) in assay mixtures and under suitable conditions that permit binding of *Mena* or *Evl* to the same or different binding partner in the control sample. In other screening assays, test and control samples include reagents that are microinjected into cells to yield an *in vivo* assay mixture. In one such method, Gst-N-Mena is microinjected into PtK_2 cells seeded to semi-confluency on Cellocate coverslips (Eppendorf, Hamburg, Germany). Labeled anti-Gst or anti-N-Mena antibodies are also injected or otherwise delivered (e.g. by microporation or liposomal transfection) into the cells. In a control sample of cells thus treated, the pattern and/or intensity of antibody labeling is detected. In the test sample, a test substance such as the ActA sequence SEPSSFEFPPPPTDEELRLA (SEQ ID NO. 12), is delivered into the cell prior to detecting the pattern and/or intensity of antibody labeling. Results of this form of assay can be readily determined based on simple qualitative observations, for example by immunofluorescence visualization of Gst-N-Mena depletion from focal contacts, or from the surface of *Listeria* or other pathogens in infected cells, confined to the test sample.

Screening for modulators of *Mena* or *Evl* expression or activity in the context of pathogenic infection is a particularly useful method, both in terms of the simplicity of the assay systems available, as well as in terms of the anticipated utility of the modulators that may be uncovered in such screens. In this context it is important to note that

the *Listeria monocytogenes* organism is only one of many diverse pathogens that rely on the induction of host cytoskeletal dynamic processes for pathogenicity. The ability to grow in a host cell cytoplasm accompanied by actin-based movement and cell-to-cell spreading has also been observed for the closely related pathogen *Listeria ivanovii* and is shared by two other groups of pathogenic bacteria, *Shigella flexneri and Rickettsia conorii and Rickettsia rickettsi* (reviewed in Chakraborty *et al. EMBO J. 14*:1314-21, 1995). In *S. flexneri,* a gene encoding an outer membrane protein variously denoted VirG or IcsA has been identified and is required for host actin recruitment to form actin halos and comets to spread from cell to cell (Pollard, *Current Biology* 5:837-40, 1995, which is incorporated herein by reference in its entirety). In addition to these pathogens which appear to share closely related actin recruitment mechanisms, there are a variety of different pathogens having apparently distinct mechanisms and/or used for host cell actin recruitment. Among these organisms, the often lethal enteropathogenic *E. coli* (EPEC) and the pathogenic enterohemorrhagic form of *E. coli* (EHEC) have the ability to induce host cell tyrosine kinases and actin accumulation leading to profound host cytoskeletal disruption (reviewed by Donnenburg *et al., J. Clin. Invest. 92*:1418-24, 1995, which is herein incorporated by reference in its entirety). In addition, diverse viral pathogens have recently been discovered to also cause profound changes in cytoskeletal dynamic processes. For example, the HIV virus spreads between cells via syncitia formation which involves both cell motility and reorganization of the cytoskeleton accompanied by condensation of F-Actin (Sylwester *et al., J. Cell. Sci. 106*:941-53, 1993, which is herein incorporated by reference in its entirety). Likewise, vaccinia virus in its intracellular enveloped form induces the formation of actin tails that are "strikingly similar" to those seen in *Listeria, Shigella and Rickettsia* infections (Cudmore *et al., Nature 378*:636-8, 1995, which is herein incorporated by reference in its entirety). Considering the diversity and widespread occurrence of pathogenic cytoskeletal induction mechanisms,

and in view of the particularly deleterious impacts of known pathogens that employ these mechanisms against humans and other mammals, the screening methods of the invention will be particularly useful to identify negative modulators of *Mena* or *Evl* expression and/or activity. Once identified, these *Mena* or *Evl* inhibitors will supplement existing tools and methods within the invention for preventing and treating such pathogenic diseases.

A variety of other screening methods are made available within the invention which rely on the construction of recombinant cell lines, ova, transgenic embryos and animals transformed to include mutant forms of the *Mena* or *Evl* genes, including dominant-negative and "knock-out" recombinants in which the activity of *Mena* or *Evl* protein is down-regulated or eliminated. Generally, *Mena* or *Evl* based recombinant polynucleotide expression constructs are provided which include a polynucleotide insert encoding a *Mena* or *Evl* protein or peptides, fusion protein or the like operably incorporated within an expression vector capable of directing expression of the polynucleotide insert in a mammalian host cell. Such constructs may include native or mutagenized forms of the *Mena* or *Evl* protein or of selected polypeptide portions of *Mena* or *Evl* such as the EVH1 functional domain. Various such constructs are provided, including constructs that incorporate polynucleotide sequences encoding the native 140 kD, 60 kD, 80 kD or 88 kD *Mena* isoforms, *Evl* or consensus sequences derived from SEQ ID NO:9. Cells transformed with these constructs may contain for example altered *Mena* coding sequences that result in the expression of a *Mena* protein that is not capable of binding to one or more of the EVH1 domain ligands of *Mena*, including ActA, zyxin and vinculin, or which has enhanced binding to profilin that results in a phenotype characterized by hyperfunctional actin polymerization. The subject cell lines and animals find uses in screening for candidate therapeutic agents capable of either substituting for a function performed by *Mena* or correcting the cellular defect caused by a defective *Mena*. In addition, the polynucleotide molecules of the present invention may be joined to reporter

genes, such as β-galactosidase or luciferase, and inserted into the genome of a suitable embryonic host cell such as an mouse embryonic stem cell by, for example, homologous recombination (for review, see Capecchi, *Trends in Genetics* 5: 70-76, 1989; which is incorporated by reference). Cells and cell lines expressing the subject molecules may then be obtained and used, for example, for screening for compounds that increase or decrease expression of the reporter gene.

In one such example discussed in more detail below, "knock-out" mice are generated by replacing the murine *Mena* or *Evl* coding region with the β-galactosidase reporter gene and the neomycin resistance gene to assess the consequences of eliminating the murine *Mena* or *Evl* protein, and to examine the tissue distribution of *Mena* or *Evl* in fetal and post-natal mice. These "Knock out" mice are useful for example as model systems for screening compounds that may developmentally, spatially and/or quantitatively alter the expression of the reporter gene. Such mice may be used to study methods to rescue homozygous mutants and as hosts to test transplant tissue for treating diseases or other conditions characterized by aberrant regulation of cytoskeletal dynamic processes.

In addition to the screening and diagnostic methods disclosed herein, the invention provides a range of therapeutic methods for preventing and/or treating diseases and other conditions that involve aberrant cytoskeletal regulation, for example pathogenic infection, traumatic neural injury and cancer. Therapeutic methods of the invention variously utilize *Mena* or *Evl* encoding polynucleotides, *Mena* or *Evl* proteins and peptides, *Mena* or *Evl* binding antibodies, *Mena* or *Evl* based expression constructs, mammalian host cells transformed to express *Mena* or *Evl* insert sequences, and other therapeutic agents generally characterized as modulators of *Mena* or *Evl* expression or activity, including triplex forming oligonucleotides, antisense polynucleotides and ribozymes that specifically target *Mena* and/or *Evl* polynucleotides.

In preferred therapeutic methods directed to the treatment of pathogenic infection and cancer, it is useful to employ agents that inhibit *Mena* or *Evl* expression or activity

and which thereby can eliminate or impair aberrant recruitment of *Mena* by pathogens, or interrupt *Mena* expression or activity associated with cancerous cell migration. In preferred therapeutic methods directed to the treatment of traumatic neural injury and other injury conditions where it is desirable to promote cell migration for neural regeneration or wound healing, it is useful to employ agents that induce or enhance *Mena* or *Evl* expression or activity. Useful agents in this context generally include agents that enhance *Mena* or *Evl* binding to its partners or otherwise enhance *Mena* or *Evl* expression or activity, for example mutant *Mena or Evl* based expression constructs that induce or enhance *Mena* or *Evl* expression or activity in targeted cells.

Therapeutic substances which can serve as inhibitors or antagonists of *Mena* or *Evl* include, but are not limited to, compounds capable of inhibiting the formation of *Mena*-profilin or *Mena*-proline-rich binding partner, compounds that reduce or inhibit the activity of *Mena*, and compounds that interfere with the expression of *Mena* protein. Such agents may include chemical compound inhibitors of *Mena*, protein or peptide *Mena* antagonists, and molecules that inhibit the expression of *Mena* such as triplex forming oligonucleotides, antisense polynucleotides, ribozymes, etc..

The use of antisense polynucleotides and their applications are described generally in, for example, Mol and Van der Krul, eds., *Antisense Nucleic Acids and Proteins Fundamentals and Applications*, New York, NY, 1992, which is incorporated by reference herein in its entirety. Suitable antisense oligonucleotides are at least 11 nucleotides in length and up to and including the upstream untranslated and associated coding sequences of *Mena*. As will be evident to one skilled in the art, the optimal length of antisense oligonucleotides is dependent on the strength of the interaction between the antisense oligonucleotides and their complementary sequence on the mRNA, the temperature and ionic environment in which translation takes place, the base sequence of the antisense oligonucleotide, the presence of secondary and tertiary structure in the mRNA and/or in the

antisense oligonucleotide and the preferred delivery mode. For example, soluble antisense oligonucleotides have been used to inhibit transcription/translation of a target gene (Ching *et al., Proc. Natl. Acad. Sci. U.S.A. 86*:10006-10010, 1989; Broder *et al., Ann. Int. Med. 113*:604-618 (1990); Loreau *et al., FEBS Letters 274*:53-56 1990; Holcenberg *et al.*, WO91/11535; U.S.S.N. 07/530,165 ("New human CRIPTO gene"); WO91/09865; WO91/04753; WO90/13641; and EP 386563, each of which is incorporated herein by reference). Suitable target sequences for antisense polynucleotides include intron-exon junctions (to prevent proper splicing), regions in which DNA/RNA hybrids will prevent transport of mRNA from the nucleus to the cytoplasm, initiation factor binding sites, ribosome binding sites, and sites that interfere with ribosome progression. A particularly preferred target region for antisense polynucleotides is the 5' untranslated region of the gene of interest, for example the *Mena* gene.

Antisense polynucleotides targeted to the *Mena* gene may also be prepared by inserting a DNA molecule containing the target DNA sequence into a suitable expression vector such that the DNA molecule is inserted downstream of a promoter in a reverse orientation as compared to the gene itself. The expression vector may then be transduced, transformed or transfected into a suitable cell resulting in the expression of antisense polynucleotides. Alternatively, antisense oligonucleotides may be synthesized using standard manual or automated synthesis techniques. Synthesized oligonucleotides may be introduced into suitable cells by a variety of means including electroporation (e.g., as described in Yang *et al., Nucl. Acids. Res. 23*:2803-2810, 1995), calcium phosphate precipitation, microinjection, poly-L-ornithine/DMSO (Dong *et al., Nucl. Acids. Res. 21*:771-772, 1993). The selection of a suitable antisense oligonucleotide administration method will be evident to one skilled in the art. With respect to synthesized oligonucleotides, the stability of antisense oligonucleotide-mRNA hybrids may be increased by the addition of stabilizing agents to the oligonucleotide. Stabilizing agents include intercalating agents that are covalently

human EST accession #T80305 (obtained from , Washington University-Merck EST Project IMAGE Consortium, LLNL). The EST was used to screen the library as described above. A murine cDNA was isolated and sequenced as previously described in detail. The cDNA (nucleotide sequence and deduced amino acid sequence in SEQ ID NO:7 and SEQ ID NO: 8) was predicted to encode a 393 amino acid protein. Based on sequence analysis the cDNA was termed Ena-VASP Like (Evl).

Alignment of Mena, Evl, human VASP, and Ena revealed two distinct blocks of similarity flanking a proline-rich core. The consensus sequence is shown in SEQ ID NO: 9. The greatest degree of amino acid identity is in amino terminal 113 amino acid of SEQ ID NO: 9, which is termed "Ena-VASP homology domain 1" (EVH1). A pair-wise comparison among the 5 sequences throughout the EVH1 domain revealed that Mena and Ena are the most similar of the four Ena-VASP family members. Serine residues aligning to two of the known cyclic nucleotide kinase-dependent phosphorylation sites in VASP (Butt et al., J. Biol. Chem. 269: 14509-14517, 1994) flank the central proline-rich region of Mena. Evl contains the amino-terminal site only, while Ena lacks both sites. The sequence GPPPPP (SEQ ID NO: 13), which mediates binding of VASP to the actin-monomer sequestering protein profilin (Reinhard et al., EMBO J. 14: 1583-1589, 1995) is present twice in Mena and once in Evl. The carboxy terminal homology domain (EVH2, spanning amino acids 507-733 in the consensus line), contains a putative G-actin binding sequence, and a conserved charge cluster.

Secondary structure analyses predict that the carboxy terminus of EVH2 is a helical region. Mena also contains a striking five amino-acid repeat region with the consensus LERER (SEQ ID NO: 10), located between the EVH1 domain and the first conserved serine phosphorylation site at amino acid 236 of SEQ ID NO: 2. The extended helical structure predicted for this repeat may function as a protein-binding interface, or serve to separate the EVH1 domain from the proline-rich core of Mena. Both the EVH2 and the LERER (SEQ ID NO: 10) repeats were predicted to form coiled-coiled domains, structures that are implicated in protein:protein interactions, by the PEPCOIL program, which employs the predictive criteria proposed by Lupas and colleagues (Lupas et al, Science 252: 1162-1164, 1991, which is incorporated herein by reference in its entirety).

The Mena cDNA was used to probe the Jackson backcross hybrid mapping panel (The Jacson Laboratory, Bar Harbor, ME) to determine the chromosomal location of the

Mena gene. Mena was localized to the mouse chromosome 1a offset 73 which is syntenic to a human chromosomal position 1Q21-23 (Stanier et al., Genomics 26: 473-478, 1995).

Example 2
Mena-Specific Antibodies

To detect Mena protein, Mena-specific antibodies were developed. DNA fragments encoding amino acids 6-170 of SEQ ID NO: 2 (N-Mena) or amino acids 440-537 of SEQ ID NO: 2 (C-Mena) were amplified from the Mena cDNA using polymerase chain reaction amplification. The fragments were each cloned into pGex2T (Smith and Johnson Gene 67: 31-40, 1988, which is incorporated herein by reference in its entirety) and transformed into a bacterial host to permit the expression of Mena-glutathione S-transferase fusion proteins. The fusion proteins were purified on a glutathione-coupled beaded agarose matrix (Glutathione SEPHAROSE, Pharmacia Biotech, Inc., Piscataway, NJ) according to the manufacturer's instructions. Rabbits were immunized and boosted with the Mena fusion proteins, and sera collected as described by Harlow and Lane in Antibodies A Laboratory Manual (Cold Spring Harbor, NY, Cold Spring Harbor Press 1988), which is incorporated herein by reference in its entirety. Affinity purified antisera raised against the peptide LKEELIDAIRQELSKSNTA (SEQ ID NO: 11) were produced by Quality Controlled Biochemicals, Inc. (Hopkins, MA). Purified platelet VASP protein was not recognized by any of the Mena antibodies tested.

Example 3
Distribution of Mena

The distribution of Mena protein isoforms during a portion of mouse embryogenesis was determined by Western blot analyses of protein from dissected heads or bodies of embryo-lysates taken from stages E10 to E15 or from lysates of whole E9 embryos. Protein extracts were from cells or dissected embryos were prepared in ice cold RIPA buffer (25 mM Tris (pH 7.5), 150 mM NaCl, 1 % NP40, 0.5% Deoxycholate, 0.1% SDS) + 1 mM PMSF, Aprotinin and 1mM $NaVO_4$. Proteins were quantified using the Pierce protein assay kit (Pierce Chemical Company, Rockford, IL).

Immunoprecipitations were performed with 1ml of 1mg/ml of head lysate using 5µl of Anti-N-Mena (from rabbit 2188) or 2188 pre-immune sera. Fifty micrograms of protein

were used for Western blotting of total cell lysates. Gel electrophoreses and Western blotting on polyvinylidene fluoride membranes (PVDF, Millipore, Bedford, MA) were performed as described by Harlow and Lane (ibid.) and according to the manufacturer's instructions. Signals were visualized using chemiluminescence (Dupont Co. (NEN Life Science Products), Wilmington, DE).

Western analysis of the dissected embryo lysates showed two bands migrating at 80 and 88 kD in all of the lysates, while a 140 kD signal was enriched in head fractions and increased in intensity from E10-E15, an interval of development during which there is rapid neurite outgrowth. In adult tissues, the 140 kD form was found only in brain extracts and not in muscle, lung, kidney, heart, liver or thymus. In addition, an approximately 60 kD Mena protein was detected in certain hematopoietic cells.

The 140 kD band was also enriched during the course of retinoic acid-induced differentiation of the P19 embryonic carcinomal stem cells into neurons (Rudnicki and McBurney, Cell Culture Methods and Induction of Differentiation of Embryonal Carcinoma Cell Lines. In Teratocarcinomas and Embryonic Stem Cells: A Practical Approach, E. Robertson, ed., Oxford, England, pp. 19-47, 1987; which is incorporated herein by reference in its entirety). Anti-Mena Western blot analyses of lysates from P19 embryonic carcinomal cells at 0,3,5, and 7 days after treatment with retinoic acid demonstrated that the 140 kD form of Mena was induced during the differentiation process. Similar results were observed using antisera directed against the amino terminus, carboxy terminus, or an affinity-purified anti-peptide antibody. Immunofluorescent staining of differentiated P19 cells with Mena-specific antibodies shows that Mena protein is concentrated in the growth cone of the neurite. Mena immunoreactivity was also observed along the length of the neurite and in the cell body.

The mobility of the 80 kD and 140 kD Mena isoforms was slower than that predicted by their sequence (60 kD and 83 kD respectively), perhaps due to structural effects of their large proline content. The Mena cDNA was translated *in vitro* to produce ^{35}S-labeled Mena protein using the TNT coupled transcription/translation system (Promega Corp, Madison, CA). The translation products were subjected to gel electrophoresis, and the gels were fixed and treated with Amplify (Amersham, Arlington Heights, IL). The fixed gels were dried and quantified using a phosphoimager (Molecular Dynamics, Sunnyvale, CA). Translation of the Mena cDNA gave rise to a protein that co-

migrates with the 80 kD band, while expression of the neural Mena cDNA (Example 4) in fibroblasts produced a signal at 140 kD in addition to the endogenous doublet at 80 kD. These data indicate that the 80 kD signal is a broadly expressed form of Mena, while the 140 kD signal is a Mena isoform enriched in, or specific to neural cell types and produced by alternative splicing. The 88 kD signal is immunoreactive with all anti-Mena antibodies tested, and may represents another splice variant, or a post-translationally modified form of Mena.

Example 4

Cloning of Neural Mena cDNAs

To clone the larger neural-enriched proteins identified as being immunologically related to Mena a mouse brain cDNA library with the Mena cDNA. The Mena cDNA was radiolabeled and used to screen a mouse brain library (Stratagene Cloning Systems, La Jolla, CA) using the methods described above. Complementary DNAs combinations of three alternately included exons spliced into the original sequence were isolated. Neural Mena^{+}, contained an exon that introduces 244 amino acids (corresponding to amino acid 239 through amino acid 482 of SEQ ID NO: 4) between amino acids 238 and 239 of (SEQ ID NO: 2). The nucleotide sequence and deduced amino acid sequences of neural Mena are shown in (SEQ ID NO: 3 and SEQ ID NO:4). Two other isoforms, neural Mena^{++} and neural Mena^{+++} contained the exon insertion described for Neural Mena^{+} and either a exon insertion between amino acids 116 and 117 of SEQ ID NO: 2. The deduced amino acid sequences of neural Mena^{++} and neural Mena^{+++} are shown in SEQ ID NO: 5 and SEQ ID NO:6, respectively.

Example 5

Phosphorylation of Mena

The presence of potential phosphorylation sites in the deduced amino acid sequence of Mena and the phosphorylation that has been demonstrated on VASP and Ena suggests that Mena is phosphorylated. The phosphorylation status of Mena was determined by immunoprecipitation. RIPA lysates from E12 embryonic heads were

immunoprecipitated using anti-N terminal Mena antibodies or pre-immune sera . The precipitates were analyzed by Western blotting as described above with the 4G10 anti-phosphotyrosine monoclonal antibody. Mena immunoprecipitates from E12 head lysates contained an anti-phosphotyrosine reactive signal that co-migrates with the 140 kD form. The 80 and 88 kD forms contained no detectable phosphotyrosine.

Mena expression was examined in the rat embryo line Rat2 (ATCC CRL 1764). The cells were grown in Dulbecco's Modified Eagle's Medium (DMEM; Life Technologies (GIBCO/BRL), Gaithersburg, MD) supplemented with 10% FCS. In Rat2 cells, Mena migrates as an 80 kD doublet. Treatment of Mena protein with purified protein kinase A (New England Biolabs, Beverly, MA) according the manufacturer's instructions caused a quantitative conversion of the 80 kD form of Mena to the slower migrating form of the doublet.

The Mena cDNA was translated *in vitro* and labeled with ^{35}S-methionine as described previously. The translation product migrated as an 80 kD doublet. Addition of protein kinase A shifted the mobility of the lower band to that of the upper, indicating that this doublet was caused by partial serine phosphorylation in the lysate.

Example 6
Subcellular Localization of Mena

The subcellular distribution of Mena in non-neural cell types was determined by immunofluorescence. Rat2 cells were grown in DMEM (Life Technologies (GIBCO/BRL), Gaithersburg, MD) supplemented with 10% FCS on glass coverslips coated with 5 μg/cm^2 fibronectin (Sigma Biosciences, St. Louis, MO). Cells were fixed with either 3% or ice-cold 4% paraformaldehyde in phosphate buffered saline (PBS) for 10 minutes. The fixed cells were permeabilized with 0.2% (v/v) Triton X-100 in PBS and stained as described (Harlow and Lane, ibid.). Mena-specific antisera was used at dilution of 1:400. Fluorescein (FITC) and Texas Red secondary antibody conjugates were purchased from Jackson Immunoresearch Laboratories, Inc., West Grove, PA) and used at a dilution of 1:100. Phosphotyrosine and neurofilaments detected using mab4G10 (Upstate Biotechnology, Inc., Lake Placid, NY), and mab 2H3D12 (obtained from Tom Jessell, Columbia University, New York, NY). Bodipy phalloidin (Molecular Probes Inc.,

uptake of the oligonucl otides by cells. Such lipids include cationic lipids used for lipofection such as N-[1-(2,3-dioleyloxy)propyl-N,N,N-trimethylammonium chloride (DOTMA) and dioleoyl phosphatidylethanolamine (DOPE). One skilled in the art may determine the particular lipid formulation or concentration that will be effective for enhancing the uptake of the oligonucleotide.

Within the methods described in detail herein, *Mena* or *Evl* inhibitors or agonists may be used in combination with other compounds that are useful, for example in chemotherapy or as antibiotics. These compounds include standard chemotherapeutic agents such as platin compounds (e.g. cisplatin) and antibiotics such as penicillin, tetracycline or antiviral agents such as protease inhibitors and the like.

Within additional aspects of the invention, the *Mena or Evl* based polynucleotide expression constructs disclosed herein are employed in methods for modulating the expression of *Mena* or *Evl*, disrupting *Mena* or *Evl* expression or inducing ectopic expression or *Mena or Evl* genes and related polynucleotide sequences in the context of gene therapeutic methods involving mammalian host cells. In preferred gene therapeutic methods of the invention, *Mena* is ectopically expressed or over expressed in mammalian cells to induce cytoskeletal changes, including for example formation of dense F-actin clusters, cell surface protrusions characterized by *Mena*-actin core structures, and filopodial extension and cytoplasmic bridge formation between neighboring cells. Introduction of the subject nucleotide sequences into cells may be accomplished *in vitro* or *in vivo* using a suitable gene therapy vector delivery system (e.g., a retroviral vector), a microinjection technique (see, for example, Tam, *Basic Life Sciences* 37:187-194, 1986, which is incorporated by reference herein in its entirety), or a transfection method (e.g., naked or liposome encapsulated DNA or RNA) (see, for example, *Trends in Genetics* 5:138, 1989; Chen and Okayama, *Biotechniques* 6:632-638, 1988; Mannino and Gould-Fogerite, *Biotechniques* 6:682-690, 1988; Kojima *et al.*, *Biochem. Biophys. Res. Comm.* 207:8-12, 1995; which are incorporated by reference herein in

their entirety). Gene transfer vectors (e.g., retroviral vectors, and the like) may be constructed wherein a polynucleotide molecule of the invention is inserted into the vector under the control of a promoter. Gene therapy may be used to correct traumatic neural injury that has resulted in loss of motor or sensory neural function. The introduction method may be chosen to achieve a transient expression of *Mena* in the host cell, or it may be preferable to achieve constitutive, tissue specific, or inducible expression.

Methods of treatment employing transformed host cells of the present invention are useful in a variety of *in vivo* settings, for example, for transplantation at sites of traumatic neural injury where motor or sensory neural activity has been lost. The polynucleotide constructs and insert sequences of the invention provide the possibility of specific gene therapy for the treatment of certain neurological disorders such as Alzheimer's disease, Huntington's disease, and Parkinson's disease, in which a population of neurons have been damaged. Representative patient populations that may benefit from transplantation include, but are not limited to, patients with hearing or vision loss due to optical or auditory nerve damage, patients with peripheral nerve damage and loss or motor or sensory neural function, individuals with diseases such as cancer or developmental defects relating to aberrant cytoskeletal dynamic function, and patients with brain or spinal cord damage from traumatic injury. In one example, donor cells fora patient having CML are obtained from a normal stem cell population in the same patient or in a donor patient and are then transformed or transduced with a mutant *Mena* or *Evl* nucleotide sequence. A preferred *Mena* mutant in this context can be designed to express a hyperfunctional EVH1 domain that has a strongly enhanced binding affinity for ligands such as zyxin or vinculin that mediate *Mena* function in cytoskeletal dynamic processes. To render this mutant effective as an antagonist of native *Mena* function in the transformed donor cells, a second mutation can be introduced in a separate functional domain, for example the proline rich, profilin binding domain, that renders the double

mutant protein incapable of binding profilin to promote actin polymerization, while it remains tightly bound to its EVH1 domain ligands and thereby prevents or impairs binding and function of endogenous *Mena* in the donor cells. The transformed cells that are rendered resistant to the motility defects and loss of contact inhibition of CML cells are then returned to the patient, for example in the manner routinely performed during bone marrow transplants. Alternatively, gene transfer may be achieved by introducing the sequences of the present invention directly to the site of the traumatic injury.

The following examples are offered by way of illustration, not by way of limitation.

Example I

Cloning of *Mena* and *Evl*

A cDNA encoding *Mena* was identified by screening a cDNA library made from mouse embryonic stem cells (Chen *et al.*, *Proc. Natl. Acad. Sci. USA 92*:7819-7823, 1994). Filter lifts of the library were prepared on nylon membranes (HYBOND, Amersham, Arlington Heights, IL). Library screening, subcloning and polymerase chain reactions were performed, and standard solutions prepared essentially as described by Sambrook *et al.* (*Molecular Clonings: A Laboratory Manual*, Cold Spring Harbor, NY, 1989, which is incorporated herein by reference in its entirety). The library was screened with a ^{32}P-labeled (Amersham, Arlington Heights, IL) Eco R1/Sph I fragment containing the first 106 codons of *Ena* (Gertler *et al.*, *Genes Dev. 9*:521-533, 1995). The filters were hybridized in a buffer containing: 1.5 x SSPE, 7% SDS, 10% PEG8000 and 1x10^6 cpm/ml of the ^{32}p-labeled *Ena* probe overnight at 42°C. The filters were washed repeatedly at 42°C in a buffer containing 2 x SSC, 1% SDS followed by washes at 60°C in a buffer containing 0.5 x SSC, 1% SDS.

Positive phage were identified by autoradiography and purified. The cDNA inserts were subcloned into pBSKSII (Stratagene Cl··.ing Systems, La Jolla, CA). The inserts were prepared and sequenced on both strands using an Applied

Biosystems DNA Sequencer model 373A (Applied Biosystems, Foster City, CA) according to the manufacturer's guidelines. Analysis of overlapping cDNAs indicated that these sequences contained an open reading frame with a predicted amino acid sequence of 541 amino acids (SEQ ID NO. I and SEQ ID NO. 2). The complete cDNA was termed Mammalian *Ena* (*Mena*). Sequence analysis was carried out using the Genetics Computer Group software package (Devereaux *et al.*, *Nuc. Acids Res. 12*: 387-395, 1994). The analysis showed significant similarity to Drosophila *Ena* on the amino acid level. Initial sequence alignments were generated using the PILEUP program and modified using the LINEUP sequence editor

The *Mena* sequence was used to search the GenBank databases at the National Center for Biotechnology Information (Bethesda, MD) using the BLAST program. Two related sequences were identified: VASP (Harmer *et al.*, *EMBO J. 14*:19-27. 1995), and human EST accession #T80305 (obtained from, Washington University-Merck EST Project IMAGE Consortium, LLNL). The EST was used to screen the library as described above. A murine cDNA was isolated and sequenced as previously described in detail. The cDNA (nucleotide sequence and deduced amino acid sequence in SEQ ID NO: 7 and SEQ ID NO. 8) was predicted to encode a 393 amino acid protein. Based on sequence analysis the cDNA was termed *Ena*-VASP Like (*Evl*).

Alignment of *Mena*, *Evl*, human VASP, and *Ena* revealed two distinct blocks of similarity flanking a proline-rich core. The consensus sequence is shown in SEQ ID NO: 9. The greatest degree of amino acid identity is in amino terminal 113 amino acid of SEQ ID NO: 9, which is termed "*Ena*-VASP homology domain 1"(EVH1). A pair-wise comparison among the 5 sequences throughout the EVH1 domain revealed that *Mena* and *Ena* are the most similar of the four *Ena*-VASP family members. Serine residues aligning to two of the known cyclic nucleotide kinase-dependent phosphorylation sites in VASP (Butt *et al.*, *J. Biol. Chem. 269*:14509-14517, 1994) flank the central proline-rich region of *Mena*. *Evl* contains the amino-terminal site only, while *Ena* lacks both sites. The sequence GPPPPP (SEQ ID NO. 13), which mediates binding of VASP to the actin-

monomer sequestering protein profilin (Reinhard *et al.*, *EMBO J. 14*:1583-1589, 1995) is present twice in *Mena* and once in *Evl*. The carboxy terminal homology domain (EVH2, spanning amino acids 507-733 in the consensus line), contains a putative G-actin binding sequence, and a conserved charge cluster.

Secondary structure analyses predict that the carboxy terminus of EVH2 is a helical region. *Mena* also contains a striking five amino-acid repeat region with the consensus LERER (SEQ ID NO: 10), located between the EVH1 domain and the first conserved serine phosphorylation site at amino acid 236 of SEQ ID NO. 2. The extended helical structure predicted for this repeat may function as a protein-binding interface, or serve to separate the EVH1 domain from the proline-rich core of *Mena*. Both the EVH2 and the LERER (SEQ ID NO. 10) repeats were predicted to form coiled-coiled domains, structures that are implicated in protein:protein interactions, by the PEPCOIL program, which employs the predictive criteria proposed by Lupas and colleagues (Lupas et al, *Science 252*:1162-1164, 1991, which is incorporated herein by reference in its entirety).

The *Mena* cDNA was used to probe the Jackson backcross hybrid mapping panel (The Jackson Laboratory, Bar Harbor, ME) to determine the chromosomal location of the *Mena* gene. *Mena* was localized to the mouse chromosome 1a offset 73 which is syntenic to a human chromosomal position 1Q21-23 (Stanier *et al.*, *Genomics 26*:473-478, 1995).

Example 2

Mena-Specific Antibodies

To detect *Mena* protein, *Mena*-specific antibodies were developed. DNA fragments encoding amino acids 6-170 of SEQ ID NO. 2 (N-Mena) or amino acids 440-537 of SEQ ID NO. 2 (C-Mena) were amplified from the *Mena* cDNA using polymerase chain reaction amplification. The fragments were each cloned into pGex2T (Smith and Johnson *Gene 67*:31-40, 1988, which is incorporated herein by reference in its entirety) and transformed into a bacterial host to permit the expression of

Mena-glutathione Transferase fusion proteins. The fusion proteins were purified on a glutathione-coupled beaded agarose matrix (Glutathione SEPHAROSE, Pharmacia Biotech, Inc., Piscataway, NJ) according to the manufacturer's instructions. Rabbits were immunized and boosted with the *Mena* fusion proteins, and sera collected as described by Harlow and Lane in *Antibodies A Laboratory Manual* (Cold Spring Harbor, NY, Cold Spring Harbor Press 1988), which is incorporated herein by reference in its entirety. Affinity purified antisera raised against the peptide LKEELIDAIRQELSKSNTA (SEQ ID NO: 11) were produced by Quality Controlled Biochemicals, Inc. (Hopkins, MA). Purified platelet VASP protein was not recognized by any of the *Mena* antibodies tested.

Example 3
Distribution of *Mena*

The distribution of *Mena* protein isoforms during a portion of mouse embryogenesis was determined by Western blot analyses of protein from dissected heads or bodies of embryo-lysates taken from stages E10 to E15 or from lysates of whole E9 embryos. Protein extracts were from cells or dissected embryos were prepared in ice cold RIPA buffer (25 mM Tris (pH 7.5), 150 mM NaCl, 1% NP40, 0.5% Deoxycholate, 0.1% SDS) + 1 mM PMSF, Aprotinin and 1 mM $NaVO_4$. Proteins were quantified using the Pierce protein assay kit (Pierce Chemical Company, Rockford, IL).

Immunoprecipitations were performed with 1 ml of 1 mg/ml of head lysate using 5 μl of Anti-N-Mena (from rabbit 2188) or 2188 pre-immune sera. Fifty micrograms of protein were used for Western blotting of total cell lysates. Gel electrophoreses and Western blotting on polyvinylidene fluoride membranes (PVDF, Millipore, Bedford, MA) were performed as described by Harlow and Lane (ibid.) and according to the manufacturer's instructions. Signals were visualized using chemiluminescence (Dupont Co. (NEN Life Science Products), Wilmington, DE).

Western analysis of the dissected embryo lysates showed two bands migrating at 80 and 88 kD in all of the

lysates, while a 140 kD signal was enriched in head fractions and increased in intensity from E10-E15, an interval of development during which there is rapid neurite outgrowth. In adult tissues, the 140 kD form was found only in brain extracts and not in muscle, lung, kidney, heart, liver or thymus. In addition, an approximately 60 kD *Mena* protein was detected in certain hematopoietic cells.

The 140 kD band was also enriched during the course of retinoic acid-induced differentiation of the P19 embryonic carcinomal stem cells into neurons (Rudnicki and McBurney, *Cell Culture Methods and Induction of Differentiation of Embryonal Carcinoma Cell Lines. In Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E. Robertson, ed., Oxford, England, pp. 19-47, 1987; which is incorporated herein by reference in its entirety). Anti-Mena Western blot analyses of lysates from P19 embryonic carcinomal cells at 0, 3, 5, and 7 days after treatment with retinoic acid demonstrated that the 140 kD form of *Mena* was induced during the differentiation process. Similar results were observed using antisera directed against the amino terminus, carboxy terminus, or an affinity-purified anti-peptide antibody. Immunofluorescent staining of differentiated P19 cells with *Mena*-specific antibodies shows that *Mena* protein is concentrated in the growth cone of the neurite. *Mena* immunoreactivity was also observed along the length of the neurite and in the cell body.

The mobility of the 80 kD and 140 kD *Mena* isoforms was slower than that predicted by their sequence (60 kD and 83 kD respectively), perhaps due to structural effects of their large proline content. The *Mena* cDNA was translated *in vitro* to produce ^{35}S-labeled *Mena* protein using the TNT coupled transcription/translation system (Promega Corp, Madison, CA). The translation products were subjected to gel electrophoresis, and the gels were fixed and treated with Amplify (Amersham, Arlington Heights, IL). The fixed gels were dried and quantified using a phosphoimager (Molecular Dynamics, Sunnyvale, CA). Translation of the *Mena* cDNA gave rise to a protein that co-migrates with the 80 kD band, while

expression of the neural *Mena* cDNA (Example 4) in fibroblasts produced a signal at 140 kD in addition to the endogenous doublet at 80 kD. These data indicate that the 80 kD signal is a broadly expressed form of *Mena*, while the 140 kD signal is a *Mena* isoform enriched in, or specific to neural cell types and produced by alternative splicing. The 88 kD signal is immunoreactive with all anti-Mena antibodies tested, and may represent another splice variant, or a post-translationally modified form of *Mena*.

Example 4

Cloning of Neural *Mena* cDNAs

To clone the larger neural-enriched proteins identified as being immunologically related to *Mena* a mouse brain cDNA library with the *Mena* cDNA. The *Mena* cDNA was radiolabeled and used to screen a mouse brain library (Stratagene Cloning Systems, La Jolla, CA) using the methods described above. Complementary DNAs combinations of three alternately included exons spliced into the original sequence were isolated. Neural *Mena*$^{+}$, contained an exon that introduces 244 amino acids (corresponding to amino acid 239 through amino acid 482 of SEQ ID NO. 4) between amino acids 238 and 239 of (SEQ ID NO. 2). The nucleotide sequence and deduced amino acid sequences of neural *Mena* are shown in (SEQ ID NO. 3 and SEQ ID NO. 4). Two other isoforms, neural *Mena*$^{++}$ and neural *Mena*$^{+++}$ contained the exon insertion described for Neural *Mena*+ and either a exon insertion between amino acids 116 and 117 of SEQ ID NO. 2. The deduced amino acid sequences of neural *Mena*$^{++}$ and neural *Mena*$^{+++}$ are shown in SEQ ID NO. 5 and SEQ ID NO.6, respectively.

Example 5

Phosphorylation of *Mena*

The presence of potential phosphorylation sites in the deduced amino acid sequence of *Mena* and the phosphorylation that has been demonstrated on VASP and *Ena* suggests that *Mena* is phosphorylated. The phosphorylation status of *Mena* was determined by immunoprecipitation. RIPA lysates from E12 embryonic heads were immunoprecipitated using anti-N terminal *Mena* antibodies or pre-immune sera. The

mab 2H3D12 (obtained from Tom Jessell, Columbia University, New York, NY). Bodipy phalloidin (Molecular Probes Inc., Eugene, OR) was used to stain F-actin, DAPI (Sigma, St. Louis, MO) was used to stain nuclei, and Vectashield mounting media (Vector Labs, Burlingame, CA) were used to in the preparation of the images. Three dimensional microscopic images were captured and processed using a Deltavision microscope and software (Applied Precision Inc., Mercer Island, WA).

Subcellular distribution of *Mena* in Rat2 cells was observed in a subdomain of Focal adhesion structures. Phosphotyrosine appeared distal to *Mena*, followed by a region of overlap, and a proximal region that contains *Mena*. Note that, unlike VASP, high levels of *Mena* are not observed along the length of actin stress fibers. The staining was specific for *Mena*, as signal was not observed in cells stained with preimmune sera, or in macrophages which contain VASP, but not *Mena*. Similar *Mena* staining was observed with both anti-N-Mena, and anti-C-Mena antibodies. The distribution of *Mena* was compared to phosphotyrosine,a marker for focal adhesions (Maher *et al.*, *Proc. Natl. Acad. Sci. USA* 82:6576-6580, 1985). *Mena* was restricted to the proximal portions of the phosphotyrosine domain in most focal contacts, at the ends of F-actin stress fibers. Therefore, *Mena* distribution overlaps with molecules that are receiving extracellular matrix-induced signals and with regions of microfilament assembly.

Example 7

EHV1 domain binding

To investigate whether the highly conserved EVH1 domain mediates an interaction with zyxin or vinculin, molecules that may recruit *Mena* to focal adhesions in fibroblasts or related structures in other cell types), the EVH1 domain was used to purify proteins from embryo cell lysates. N-Mena-Gst, the Gst-fusion protein containing amino acids 6-170 of *Mena* (described in Example 2) encompasses the EVH1 domain. Five micrograms of N-*Mena*-Gst or Gst alone was immobilized on a glutathione-coupled beaded agarose matrix (Glutathione SEPHAROSE, Pharmacia Biotech, Inc., Piscataway,

NJ) according to the manufacturer's instructions. The N-Mena matrix was mixed with 1 ml of 1 mg/ml of RIPA lysates of E12 heads, or human erythrocytic leukemia cells (HEL) (described above) and incubated overnight at 4°C. Samples were washed three times in RIPA, and bound proteins analyzed by Western blot analysis with antibodies to zyxin (obtained from Dr. Mary Beckerle University of Utah), vinculin or the Ack kinase (Santa Cruz Biotechnology Inc., Santa Cruz, CA), which contains a similar proline-rich motif. The antibodies were used at a 1:100 dilution. The N-Mena matrix retained more than 50% of the total endogenous zyxin, and detectable amounts of vinculin. Ack was not recovered detectably, indicating that the motif in Ack lacks some feature critical for binding *in vivo,* or that the sequence is not accessible. These results indicate that N-Mena is capable of binding to zyxin and vinculin.

To test the ability of *Mena* to bind ActA, PtK_2, potoroo kidney epithelial cells, (ATCC CCL 56) were seeded in Modified Essential Medium (MEM; Life Technologies (GIBCO/BRL), Gaithersburg, MD) supplemented with 10% FCS, glutamine and nonessential amino acids to semi confluency onto CELLocate coverslips (Eppendorf, Hamburg, Germany) two days prior to microinjection. Purified Gst-N terminal *Mena* fusion protein was microinjected into the cytoplasm of the cells and GST alone served as a control. After injection, the cells were returned to the incubator for 15 to 30 minutes before fixation.

For the detection of microinjected proteins directed to subcellular structures, an extraction of the cytoplasm before fixation was performed by washing the cells with MES-buffer (0.1 M MES, 4 % PEG 6000 (w/v), 1 mM $MgCl_2$ (pH 7.6)) twice followed by a two minute incubation with MES-buffer with 0.2% Triton X-100. After another wash with MES-buffer, cells were fixed with paraformaldehyde, and then incubated 30 minutes each with primary and fluorescently labeled secondary antibodies and phalloidin. Microinjected Gst-N terminal *Mena* fusion protein was detected using polyclonal anti-GST antibodies. Fluorescence photomicroscopy was carried out on a

Zeiss Axiophot with appropriate filter sets for epifluorescence detection of FITC and rhodamine signals. The microinjection of N-Mena-Gst fusion protein into PtK_2 cells demonstrated that the N-*Mena* sequences directed the fusion protein to focal adhesions. When Gst alone was injected, diffuse signal was observed throughout the cell.

A peptide corresponding to amino acids 293-312 of the ActA sequence (SEPSSFEFPPPPTDEELRLA, SEQ ID NO. 12) was also injected into PtK_2 cells. The ActA peptide (SEQ ID NO. 12) was first coupled to ovalbumin as described previously (Pistor *et al.*, *Curr. Biol.* 5:517-525, 1995; which is incorporated herein by reference in its entirety), and the conjugate was microinjected along with rhodamine-coupled BSA as a marker protein. The cells were then returned to the incubator for 15 to 30 minutes before fixation.

Microinjection of the ActA peptide (SEQ ID NO. 12) was resulted in the depletion of the endogenous *Mena* protein from focal contacts in the injected cells. In contrast, *Mena* distribution was not affected by injection of a peptide corresponding to an unrelated portion of ActA. However, the distribution of vinculin was not altered by the injected peptide, confirming that the effect is specific. Taken together, these data indicate that the N-terminal portion of *Mena*, which contains the EVH1 domain, can direct its proper localization to focal contacts via direct protein:protein interactions with zyxin, vinculin or other focal adhesion proteins containing an ActA-like motif. These proteins contain the sequence FPPPP (SEQ ID NO. 17) suggesting that this motif may comprise the core recognition site in EVH1 ligands.

Example 8

Mena Modulates F-actin Dynamics

To examine whether increased concentration of *Mena* protein might stimulate actin nucleation and polymerization, a retroviral vector was used to drive increased levels, or ectopic expression of the different *Mena* isoforms in Rat2 cells. Rat2 cells were grown under conditions described previously. Retroviruses were constructed by inserting full

length *Mena* cDNAs (*Mena*, neural *Mena*, Isoform 1 or Isoform 2) into the pBABE vector (Morgenstern and Land, *Nuc. Acids Res.* 18:3587-3596, 1990, which is incorporated herein by reference in its entirety), packaged and used to infect fibroblasts as described Morgenstern and Land (ibid.).

Cells were immunostained to determine the distribution of *Mena* and F-actin three days after infection with the parental virus or viruses programmed to express the various *Mena* isoforms. Expression of the 80 kD form of *Mena* caused the formation of localized clusters of *Mena* and F-actin. More dramatic results were observed when any of the three neural variants of *Mena* were used.

The over-expression of neural *Mena* was particularly dramatic. As expected, individual cells in the experimental population expressed varying degrees of neural *Mena* as judged by fluorescence intensity. Similar increases in *Mena* immunofluorescence intensity across the population were observed with the 80 kD form, or any of the three neural *Mena* variants. Western analysis indicated that, over the whole population, the cumulative amount of ectopic neural *Mena* protein produced was roughly equivalent to the endogenous content of the 80 kD *Mena* isoform. About 25% of cells had detectable levels of *Mena* immunoreactivity outside focal contacts. These cells often contained concentrated pools of immunoreactivity that overlapped with dense accumulations of F-actin. Projection of a three dimensional rendering of the image at a 45° angle indicated that the *Mena*-actin structures were protuberances from the cell surface. Scanning electron microscopy confirmed the presence of cell-surface projections on a subset of cells from a similarly infected pool, but not in the corresponding control. An optical section taken at the plane of cell-substratum contact showed a relatively normal distribution of *Mena*, mainly in focal contacts and at the cell periphery, and F-actin, mainly in typical stress fibers. An optical section taken 3.8 μM above the substratum contact indicated the *Mena*-induced projections extend above the top of the nuclei. Comparison of the *Mena* and F-actin staining at this level indicated that *Mena* is distributed at the periphery

of the F-actin, potentially at the site of F-actin polymerization and nucleation in these projections. These data indicate that, when ectopically expressed in fibroblasts, the neural form of *Mena* can direct the formation of cell surface projections.

Example 9

Mena Interacts with Profilin and SH3

The ability of *Mena* to interact with SH3 and profilin binding was determined by using assays were described by Reinhard *et al.*(ibid., 1995) and Weng *et al.* (*J. Biol. Chem.* 268:14956-14963, 1993), which are incorporated herein by reference in their entirety). *Mena* protein was labeled with ^{35}S-methionine by *in vitro* using the TNT coupled transcription/translation system (Promega Corp, Madison, CA) according to the manufacturer's instruction.

Briefly, profilin was coupled to a NHS-activated agarose matrix (NHS-activated HiTrap-column, Pharmacia Biotech, Inc., Piscataway, NJ) following the manufacturer's instructions. Chromatography steps were carried out at 4°C. Radiolabeled *Mena* protein was loaded onto the column in the presence or absence of 1 mg/ml GPPPPP (SEQ ID NO. 13). Proteins were eluted from the column with buffer B (50 mM Tris-HCl (pH 7.2), 1 mM DTE, 5 mM $MgCl_2$, 150 mM NaCl, 0.1% BSA) with 5 mg/ml poly-L-proline (1-10 kD, Sigma, St. Louis, MO).. The eluates were analyzed by SDS-PAGE and autoradiography. Autoradiography demonstrated that 83% of the input *Mena* was retained on the profilin-SEPHAROSE column as determined by phosphoimager analysis of the gel. Equal binding of the phospho- and dephospho forms of *Mena* was observed. In the presence of the competitor peptide, the amount of *Mena* retained was reduced to 13%.

Mena-profilin binding was also assessed by incubating 5 x 10^{15} M of *Mena* with increasing concentrations of profilin to determine the % of *Mena* bound. In this assay, the profilin-SEPHAROSE matrix was diluted with Sepharose to create columns bound with known, increasing profilin concentrations. The 5 x 10^{15}M of *in vitro* translated, radiolabeled *Mena* was mixed with profilin-Sepharose matrix and

unbound *Mena* was wash d from the column. The bound *Mena* was quantified by scintillation analysis. This analysis demonstrated that the *Mena* binding is saturabl .

Mena binding was assayed for Gst-AblSH3, GstScrSH3 (described by Gertler *et al.*, ibid., 1995; which is incorporated herein by reference in its entirety). The Gst fusions proteins were each bound to glutathione-SEPHAROSE as described previously. The SH3-SEPHAROSE matrix was then gel electrophoresis and autoradiography. Gst alone was used as a control, and as expected, no binding to Gst was observed. The AblSH3 retained 21 % and 14% of the input dephospho-, or phosphorylated input *Mena*, respectively. The SrcSH3 retained 8% and 3% of the dephospho-, or phosphorylated *Mena*, respectively. In both cases, the dephosphorylated form of *Mena* was more efficiently purified from the lysate.

The Gst-SH3 fusion proteins were also used to purify *Mena* from RIPA head lysates. The Gst-fusion proteins were each bound to the glutathione-SEPHAROSE matrix as described above. RIPA head lysates were added to the matrix. The matrix was washed, and the bound protein was eluted and analyzed. Using this method both the broadly distributed and neural *Mena* forms were purified. These results indicate that a subset of interactions mediated by the proline-rich core of *Mena* could be modulated by serine-threonine phosphorylation.

Example 10

Mena Localization in *Listeria* Infection

To determine if *Mena* is involved in the microfilament assembly required for Listeria motility, the distribution of *Mena* was examined in infected cells. *L. monocytogenes* serotype 1/2a EGD were grown in brain-heart infusion (BHI) broth (DIFCO Laboratories, Inc., Detroit, MI) at 37°C with aeration. For infection experiments PtK_2 cells were grown on coverslips, and bacteria from an overnight culture were added directly to the culture medium at a dilution of 1:200. After 1-2 hours the plates were extensively washed with fresh medium containing 25 μg/ml gentamicin to kill xtracellular bacteria. After further incubation for 2-3 hours the coverslips were washed and

processed for immunofluorescence as described above. Immunofluorescence demonstrated that *Mena* is depleted from focal contacts and recruited to the bacterial surface upon infection with *Listeria monocytogenes*. Analogous to its appearance in focal adhesions, *Mena* distribution overlaps with the pole of the bacterium associated with the formation of new F-actin. The Gst-N-Mena was microinjected into PtK_2 cells. The cells were treated as described above, except that after infection, the cells were allowed to recover for at least two hours. After infection of the injected cells with Listeria, the exogenous *Mena* protein was recruited to the surface of the bacterium. These results implicate the EVH1 domain of *Mena* and VASP in the ActA-dependent redistribution of these proteins to the bacterial surface.

Example 11

Construction of Knock out Mice and Immortomouse *Mena*$^-$ Mice

Knock-out mice in which the murine *Mena* coding sequence was replaced with the β-galactosidase gene and the neomycin resistance gene (neo) were generated i) to assess the consequences of eliminating the murine *Mena* protein during mouse development, ii) to permit examination of the expression pattern of *Mena* in embryonic mice, iii) to generate *Mena*$^-$ cell lines, and iv) to cross the mice with mice carrying oncogenes to study the effects of such double mutants. Genomic *Mena* sequences used for these knock-out mice were obtained from the 129/Sv mice so that the homologous recombination could take place in a congenic background in 129/Sv mouse embryonic stem cells. *Mena* genomic clones were isolated from a genomic library prepared from 129/Sv mice (Zhuang *et al.*, *Cell* 79:875-884, 1994; which is incorporated herein by reference in its entirety) using a random-primed *Mena* cDNA probe. Plasmid pSAβGeolox2DTA contains the β-galactosidase/neomycin resistance gene fusion (βGeo) and the *Diptheria* toxin gene under the control of the PGK promoter in a pBLUESCRIPT (Stratagene Cloning Systems, La Jolla, CA) vector backbone was used to create the targeting construct. A 10 kb 5' *Mena* genomic fragment, the 4 kb cytoplasmic βGeo gene and the 1 kb fragment containing the genomic 3' untranslated sequence of

Mena was inserted 5' of the Diptheria toxin expression cassette in the pSAβGeolox2DTA vector backbone.

The targeting construct was linearized at a unique restriction site in the vector backbone and was transfected by electroporation into mouse embryonic stem (ES) cells. A 129/Sv derived ES cell line, AK-7 described by Zhuang *et al.* (ibid.) was used for electroporation. These ES cells were routinely cultured on mitomycin C-treated (Sigma) SNL 76/7 cells (feeder cells) as described by McMahon and Bradley (*Cell* 62:1073-1085, 1990; which is incorporated herein by reference in its entirety) in culture medium containing high glucose DMEM supplemented with 15% fetal bovine serum (Hyclone) and 0.1 μM (micromolar) β-mercaptoethanol. To prepare the targeting construct for transfection, 25 g (micrograms) of the targeting construct was linearized by digestion with Not I, phenol-chloroform extracted, and ethanol precipitated. The linearized vector was then electroporated into 1-2 x 10^7 AK-7 (ES) cells. The electroporated cells were seeded onto three 10-cm plates with one plate receiving 50% of the electroporated cells and the remaining two plates each receiving 25% of the electroporated cells. After 24 hours, G418 was added to each of the plates to a final concentration of 300 μg/ml (micrograms per milliliter). The culture medium for each plate was changed every day for the first few days, and then changed as needed after selection had occurred. After 10 days of selection, a portion of each colony was picked microscopically with a drawn micropipette, and was directly analyzed by PCR as described by Joyner *et al.* (*Nature* 338:153-156, 1989; which is incorporated herein by reference in its entirety). Briefly, PCR amplification was performed as described (Kogan *et al.*, *New England J. Med.* 317:985-990, 1987; which is incorporated herein by reference in its entirety) using 40 cycles of 93°C for 30 seconds, 57°C for 30 seconds, and 65°C for 3 minutes. To detect the wild-type allele, primers MF and MR (SEQ ID NOS. 14 and 15, respectively) were used in the PCR reaction, to detect the mutant *Mena* allele, primers BPAF and MR (SEQ ID NOS. 16 and 15, respectively) were used in the PCR reaction. Positive

colonies, identified by PCR, were subcloned into 4-well plates, expanded into 60 mm plates and frozen into 2-3 ampules. Among the clones that were selected for both G418-resistance (positive selection for *neo* gene expression) 95% of the population contained correctly targeted integration of the vector into the murine *Mena* locus.

To generate chimeric mice, each positive clone was thawed and passaged once on feeder cells. The transfected cells were trypsinized into single cells, and blastocysts obtained from C57BL/6J mice were injected with approximately 15 cells. The injected blastocysts were then implanted into pseudopregnant mice (C57BL/6J x CBA). Six male chimeras arose from the injected blastocysts. All of the male chimeras gave germ-line transmission at a high rate as determined by the frequency of agouti coat color transmission to their offspring (F1) in a cross with C57BL/6J female mice. Since 50% of the agouti coat color offspring (F1) should represent heterozygous mutants, their genotypes were determined by Southern blot analysis. Briefly, genomic DNA prepared from tail biopsies was digested with Eco R1 and probed with the a 3 kb 5' genomic sequence flanking the targeting construct. This probe detects a 16 kb fragment from the wild-type allele and a 10 kb fragment from the mutant allele. Therefore, a Southern analysis would show a single 16 kb band for a wild-type mouse, 16 kb and 10 kb fragments for a heterozygous mouse, and a single 10 kb band for a homozygous mutant mouse. The resulting offspring (F1), heterozygous (+/-) mice, were mated with sibling heterozygous mice to give rise to the homozygous (-/-) mutant mice.

To study *Mena* expression patterns in embryonic mice, chimeric mice or F1 heterozygous progeny from the chimera x C57B/6J mating were crossed with C57B/6J. Litters resulting from these crosses were harvested from pregnant females and stained for β-galactosidase activity. The embryos were dissected away from all the extra-embryonic tissue and the yolk sac was reserved for DNA analysis. The embryos were fixed for one hour in a Fix solution containing (0.1 M phosphate buffer containing 0.2% glutaraldehyde, 2%

formaldehyde, 5 mM EGTA (pH 7.3), 2 mM $MgCl_2$). The fixing solution was removed by three thirty-minut rinses with rinse solution (0.1 M phosphate buffer (pH 7.3) containing 2 mM $MgCl_2$, 0.1% sodium deoxycholate, 0.2% NP-40). The fixed embryos were stained overnight in the dark in rinse solution containing 1 mg/ml X-gal, 5 mM sodium ferricyanide, 5 mM sodium ferrocyanide. After staining, the embryos were rinsed with PBS and stored in the Fix solution before preparation for examination. Examination of stained tissue from fetal and postnatal mice heterozygous for the mutation demonstrated *Mena* expression pattern in the nervous system, somites, muscle tissue, neural crest cells and in the gut.

To generate mice from which *Mena*$^-$ cell lines may be derived, a heterozygous *Mena* knock-out mouse was crossed with an IMMORTOMOUSE (Charles River Laboratories, Wilmington, MA). IMMORTOMOUSE is a mouse carrying a H-2Kb-tsA58 SV40 large T antigen transgene. The progeny of the cross were subjected to PCR analysis as generally described above to identify progeny carrying the transgene and are heterozygous for the *Mena* knockout gene. The progeny carrying both the transgene and the *Mena* knockout gene were back-crossed to the *Mena*$^-$ heterozygotes. The progeny of the back-cross were subjected to PCR analysis to identify homozygous *Mena*$^-$ progeny carrying the transgene. Cells from the resulting mice may be immortalized by culturing the cells at 33°C in the presence of interferon.

Those with ordinary skill in the art will appreciate that other embodiments and variations of the invention are possible which employ the same inventive concepts described above. Therefore, the invention is not to be limited except by the above description, but is to be determined in scope by the claims which follow.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: Patent In Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
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(2) INFORMATION FOR SEQ ID NO. 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2172 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mus musculus

(vii) IMMEDIATE SOURCE:
(B) CLONE: Mena

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 140..1765

(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 1:

TCGAGATTTA CCGTAACCCG CCCGCCGGAG CCGGACCCGC CTCTGCTCGC GCGTCCCCGC 60

```
GGCGTCCGCC TCCCCGCCTG AGAAGAGACC CTCCGCTCGG CGGCTCCCGC GCCGGGGAAG 120

CCTCGGCGCC GCCGGCACC ATG AGT GAA CAG AGT ATC TGT CAG GCA AGA GCT  172
                     Met Ser Glu Gln Ser Ile Cys Gln Ala Arg Ala
                      1               5                  10

GCT GTG ATG GTC TAT GAT GAT GCC AAT AAG AAG TGG GTG CCA GCT GGT  220
Ala Val Met Val Tyr Asp Asp Ala Asn Lys Lys Trp Val Pro Ala Gly
            15                  20                  25

GGC TCA ACT GGG TTC AGC AGA GTA CAT ATA TAT CAC CAT ACA GGC AAC  268
Gly Ser Thr Gly Phe Ser Arg Val His Ile Tyr His His Thr Gly Asn
        30                  35                  40

AAC ACA TTC AGA GTT GTG GGC AGA AAG ATT CAA GAC CAT CAG GTT GTG  316
Asn Thr Phe Arg Val Val Gly Arg Lys Ile Gln Asp His Gln Val Val
    45                  50                  55

ATA AAC TGT GCC ATT CCT AAA GGG CTG AAG TAC AAT CAA GCT ACA CAG  364
Ile Asn Cys Ala Ile Pro Lys Gly Leu Lys Tyr Asn Gln Ala Thr Gln
60                  65                  70                  75

ACT TTC CAC CAA TGG AGG GAT GCT AGA CAG GTG TAT GGT CTC AAC TTT  412
Thr Phe His Gln Trp Arg Asp Ala Arg Gln Val Tyr Gly Leu Asn Phe
                80                  85                  90

GGC AGC AAA GAG GAT GCC AAT GTC TTC GCA AGT GCC ATG ATG CAT GCC  460
Gly Ser Lys Glu Asp Ala Asn Val Phe Ala Ser Ala Met Met His Ala
            95                  100                 105

TTA GAA GTG TTA AAT TCA CAG GAA GCA GGG CCA ACA TTG CCT AGA CAA  508
Leu Glu Val Leu Asn Ser Gln Glu Ala Gly Pro Thr Leu Pro Arg Gln
        110                 115                 120

AAT TCA CAG CTA CCT GCT CAA GTT CAA AAT GGC CCA TCC CAA GAA GAG  556
Asn Ser Gln Leu Pro Ala Gln Val Gln Asn Gly Pro Ser Gln Glu Glu
    125                 130                 135

CTG GAA ATC CAG AGA AGG CAA CTG CAA GAA CAG CAG CGA CAG AAG GAA  604
Leu Glu Ile Gln Arg Arg Gln Leu Gln Glu Gln Gln Arg Gln Lys Glu
140                 145                 150                 155

CTG GAG AGG GAA AGA ATG GAG AGG GAA AGG TTG GAG AGA GAA CGA CTA  652
Leu Glu Arg Glu Arg Met Glu Arg Glu Arg Leu Glu Arg Glu Arg Leu
                160                 165                 170

GAA CGA GAG AGG CTA GAG AGG GAG CGC CTG GAA CAA GAG CAG CTG GAG  700
Glu Arg Glu Arg Leu Glu Arg Glu Arg Leu Glu Gln Glu Gln Leu Glu
            175                 180                 185

CGG CAG CGG CAG GAA AGG GAG CAC GTG GAG CGG CTG GAG AGG GAG AGG  748
Arg Gln Arg Gln Glu Arg Glu His Val Glu Arg Leu Glu Arg Glu Arg
        190                 195                 200

CTG GAG CGC CTG GAG CGA GAG AGG CAG GAG CGG GAG CGA GAG CGC CTG  796
Leu Glu Arg Leu Glu Arg Glu Arg Gln Glu Arg Glu Arg Glu Arg Leu
    205                 210                 215
GAG CAG CTG GAG CGG GAG CAA GTG GAG TGG GAG CGA GAG CGC AGA ATG  844
Glu Gln Leu Glu Arg Glu Gln Val Glu Trp Glu Arg Glu Arg Arg Met
220                 225                 230                 235

TCC AAT GCT GCT GCC CCT GCC TCT GCG GAG ACC CCT CTA AAT CCT GAG  892
Ser Asn Ala Ala Ala Pro Ala Ser Ala Glu Thr Pro Leu Asn Pro Glu
                240                 245                 250
```

```
CTG GGA GAC TCC TCT GCT TCC GAG CCA GGC TTG CAG GCA GCC TCT CAG    940
Leu Gly Asp Ser Ser Ala Ser Glu Pro Gly Leu Gln Ala Ala Ser Gln
            255                 260                 265

CCG GCC GAG TCG CCA ACC CCA CAG GGC CTT GTC TTG GGA CCA CCT GCA    988
Pro Ala Glu Ser Pro Thr Pro Gln Gly Leu Val Leu Gly Pro Pro Ala
        270                 275                 280

CCT CCG CCA CCA CCC CCT CTC CCA TCA GGC CCT GCC TAC GCC TCA GCA   1036
Pro Pro Pro Pro Pro Pro Leu Pro Ser Gly Pro Ala Tyr Ala Ser Ala
    285                 290                 295

CTT CCT CCT CCC CCA GGA CCC CCT CCA CCA CCT CCA CTG CCA TCC ACT   1084
Leu Pro Pro Pro Pro Gly Pro Pro Pro Pro Pro Pro Leu Pro Ser Thr
300                 305                 310                 315

GGT CCT CCT CCT CCA CCC CCT CCA CCA CCC CCT CTT CCT AAT CAA GCT   1132
Gly Pro Pro Pro Pro Pro Pro Pro Pro Pro Pro Leu Pro Asn Gln Ala
                320                 325                 330

CCT CCC CCT CCT CCC CCA CCT CCT GCC CCT CCC CTC CCC GCA TCT GGA   1180
Pro Pro Pro Pro Pro Pro Pro Pro Ala Pro Pro Leu Pro Ala Ser Gly
            335                 340                 345

ATT TTC TCT GGA TCC ACG TCA GAA GAC AAT CGC CCT TTA ACT GGA CTT   1228
Ile Phe Ser Gly Ser Thr Ser Glu Asp Asn Arg Pro Leu Thr Gly Leu
        350                 355                 360

GCA GCT GCA ATT GCG GGA GCA AAA CTT AGG AAA GTG TCC CGG GTG GAG   1276
Ala Ala Ala Ile Ala Gly Ala Lys Leu Arg Lys Val Ser Arg Val Glu
    365                 370                 375

GAT GGC TCT TTC CCA GGT GGA GGG AAT ACT GGG AGT GTG AGC TTG GCC   1324
Asp Gly Ser Phe Pro Gly Gly Gly Asn Thr Gly Ser Val Ser Leu Ala
380                 385                 390                 395

TCA TCC AAA GCA GAC GCT GGG CGT GGG AAT GGA CCT CTT CCT CTA GGG   1372
Ser Ser Lys Ala Asp Ala Gly Arg Gly Asn Gly Pro Leu Pro Leu Gly
                400                 405                 410

GGT AGT GGC TTA ATG GAA GAA ATG AGT GCC CTG CTG GCC AGG AGG AGA   1420
Gly Ser Gly Leu Met Glu Glu Met Ser Ala Leu Leu Ala Arg Arg Arg
            415                 420                 425

AGA ATT GCT GAG AAG GGA TCA ACA ATA GAA ACA GAA CAA AAG GAA GAC   1468
Arg Ile Ala Glu Lys Gly Ser Thr Ile Glu Thr Glu Gln Lys Glu Asp
        430                 435                 440

AGA AAT GAA GAT GCA GAG CCT ATA ACT GCT AAG GCC CCA TCA ACA AGT   1516
Arg Asn Glu Asp Ala Glu Pro Ile Thr Ala Lys Ala Pro Ser Thr Ser
    445                 450                 455

ACA CCT GAA CCA ACC AGA AAA CCT TGG GAA AGA ACA AAC ACA ATG AAC   1564
Thr Pro Glu Pro Thr Arg Lys Pro Trp Glu Arg Thr Asn Thr Met Asn
460                 465                 470                 475

GGC AGT AAG TCA CCT GTC ATC TCC AGA CCC AAA TCC ACA CCT TCA TCA   1612
Gly Ser Lys Ser Pro Val Ile Ser Arg Pro Lys Ser Thr Pro Ser Ser
                480                 485                 490

CAG CCA AGT GCC AAT GGA GTC CAG ACA GAA GGC CTT GAC TAT GAC AGG   1660
Gln Pro Ser Ala Asn Gly Val Gln Thr Glu Gly Leu Asp Tyr Asp Arg
            495                 500                 505

CTG AAG CAG GAC ATT TTA GAT GAG ATG AGA AAA GAG CTG GCA AAG CTG   1708
Leu Lys Gln Asp Ile Leu Asp Glu Met Arg Lys Glu Leu Ala Lys Leu
        510                 515                 520
```

```
AAG GAG GAG CTT ATT GAC GCA ATC AGG CAG GAG CTG AGC AAG TCG AAC    1756
Lys Glu Glu Leu Ile Asp Ala Ile Arg Gln Glu Leu Ser Lys Ser Asn
    525                 530                 535

ACT GCA TAAAGAAGCA AACTACGGAG GGGCAGGACT TGAATCTGGA GAAAACAAAA    1812
Thr Ala
540

ATTCCTACAA ACAACTCTTA ACCCCCAAAC TTTTAAGCTG TAAGAAGAAA ATGGATACAC 1872

AGTCAGGAGG GAAGCCGTCA ACCTCTGAAA GCCTCAGACA GTGACTCTGG CGATCAGCTG 1932

TCCCCTCAGT GTGCTGCTTT ATTCTGTCTG ACCTTTACCA CAGGATGGAG AATGATATTG 1992

GAGTTCCCTT AGCAGTACTA AACCCGTCAG GCAAGATCAC CGTGCATTGA AATATTTTCA 2052

TGTCTAGATG AGTCTGCACG TTTTCCATAA TCCATTGCTA AAATAAAGAC GAGAAAGGGT 2112

AAATCTCGAG GAATTCATAA TTTTTTCCTC CAGATCCTCT AGAGTCCTGT TTCCTGTGTG 2172
```

(2) INFORMATION FOR SEQ ID NO.2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 541 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 2:

```
Met Ser Glu Gln Ser Ile Cys Gln Ala Arg Ala Ala Val Met Val Tyr
  1               5                  10                  15

Asp Asp Ala Asn Lys Lys Trp Val Pro Ala Gly Gly Ser Thr Gly Phe
             20                  25                  30

Ser Arg Val His Ile Tyr His His Thr Gly Asn Asn Thr Phe Arg Val
         35                  40                  45

Val Gly Arg Lys Ile Gln Asp His Gln Val Val Ile Asn Cys Ala Ile
     50                  55                  60

Pro Lys Gly Leu Lys Tyr Asn Gln Ala Thr Gln Thr Phe His Gln Trp
 65                  70                  75                  80

Arg Asp Ala Arg Gln Val Tyr Gly Leu Asn Phe Gly Ser Lys Glu Asp
                 85                  90                  95

Ala Asn Val Phe Ala Ser Ala Met Met His Ala Leu Glu Val Leu Asn
            100                 105                 110

Ser Gln Glu Ala Gly Pro Thr Leu Pro Arg Gln Asn Ser Gln Leu Pro
        115                 120                 125

Ala Gln Val Gln Asn Gly Pro Ser Gln Glu Glu Leu Glu Ile Gln Arg
    130                 135                 140

Arg Gln Leu Gln Glu Gln Gln Arg Gln Lys Glu Leu Glu Arg Glu Arg
145                 150                 155                 160

Met Glu Arg Glu Arg Leu Glu Arg Glu Arg Leu Glu Arg Glu Arg Leu
                165                 170                 175
```

```
Glu Arg Glu Arg Leu Glu Gln Glu Gln Leu Glu Arg Gln Arg Gln Glu
            180                 185                 190

Arg Glu His Val Glu Arg Leu Glu Arg Glu Arg Leu Glu Arg Leu Glu
        195                 200                 205

Arg Glu Arg Gln Glu Arg Glu Arg Glu Arg Leu Glu Gln Leu Glu Arg
    210                 215                 220

Glu Gln Val Glu Trp Glu Arg Glu Arg Arg Met Ser Asn Ala Ala Ala
225                 230                 235                 240

Pro Ala Ser Ala Glu Thr Pro Leu Asn Pro Glu Leu Gly Asp Ser Ser
                245                 250                 255

Ala Ser Glu Pro Gly Leu Gln Ala Ala Ser Gln Pro Ala Glu Ser Pro
            260                 265                 270

Thr Pro Gln Gly Leu Val Leu Gly Pro Pro Ala Pro Pro Pro Pro Pro
        275                 280                 285

Pro Leu Pro Ser Gly Pro Ala Tyr Ala Ser Ala Leu Pro Pro Pro Pro
    290                 295                 300

Gly Pro Pro Pro Pro Pro Pro Leu Pro Ser Thr Gly Pro Pro Pro Pro
305                 310                 315                 320

Pro Pro Pro Pro Pro Pro Leu Pro Asn Gln Ala Pro Pro Pro Pro Pro
                325                 330                 335

Pro Pro Pro Ala Pro Pro Leu Pro Ala Ser Gly Ile Phe Ser Gly Ser
            340                 345                 350

Thr Ser Glu Asp Asn Arg Pro Leu Thr Gly Leu Ala Ala Ala Ile Ala
        355                 360                 365

Gly Ala Lys Leu Arg Lys Val Ser Arg Val Glu Asp Gly Ser Phe Pro
    370                 375                 380

Gly Gly Gly Asn Thr Gly Ser Val Ser Leu Ala Ser Ser Lys Ala Asp
385                 390                 395                 400

Ala Gly Arg Gly Asn Gly Pro Leu Pro Leu Gly Gly Ser Gly Leu Met
                405                 41                  415

Glu Glu Met Ser Ala Leu Leu Ala Arg Arg Arg Arg Ile Ala Glu Lys
            420                 425                 430

Gly Ser Thr Ile Glu Thr Glu Gln Lys Glu Asp Arg Asn Glu Asp Ala
        435                 440                 445

Glu Pro Ile Thr Ala Lys Ala Pro Ser Thr Ser Thr Pro Glu Pro Thr
    450                 455                 460

Arg Lys Pro Trp Glu Arg Thr Asn Thr Met Asn Gly Ser Lys Ser Pro
465                 470                 475                 480

Val Ile Ser Arg Pro Lys Ser Thr Pro Ser Ser Gln Pro Ser Ala Asn
                485                 490                 495

Gly Val Gln Thr Glu Gly Leu Asp Tyr Asp Arg Leu Lys Gln Asp Ile
            500                 505                 510

Leu Asp Glu Met Arg Lys Glu Leu Ala Lys Leu Lys Glu Glu Leu Ile
        515                 520                 525
```

Asp Ala Ile Arg Gln Glu Leu Ser Lys Ser Asn Thr Ala
530 535 540

2) INFORMATION FOR SEQ ID NO. 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2898 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mus musculus

(vii) IMMEDIATE SOURCE:
(B) CLONE: Neural Mena

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 140..2491

(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 3:

```
TCGAGATTTA CCGTAACCCG CCCGCCGGAG CCGGACCCGC CTCTGCTCGC GCGTCCCCGC   60
GGCGTCCGCC TCCCCGCCTG AGAAGAGACC CTCCGCTCGG CGGCTCCCGC GCCGGGGAAG  120
CCTCGGCGCC GCCGGCACCA TGAGTGAACA GAGTATCTGT CAGGCAAGAG CTGCTGTGAT  180
GGTCTATGAT GATGCCAATA AGAAGTGGGT GCCAGCTGGT GGCTCAACTG GGTTCAGCAG  240
AGTACATATA TATCACCATA CAGGCAACAA CACATTCAGA GTTGTGGGCA GAAAGATTCA  300
AGACCATCAG GTTGTGATAA ACTGTGCCAT TCCTAAAGGG CTGAAGTACA ATCAAGCTAC  360
ACAGACTTTC CACCAATGGA GGGATGCTAG ACAGGTGTAT GGTCTCAACT TTGGCAGCAA  420
AGAGGATGCC AATGTCTTCG CAAGTGCCAT GATGCATGCC TTAGAAGTGT TAAATTCACA  480
GGAAGCAGGG CCAACATTGC CTAGACAAAA TTCACAGCTA CCTGCTCAAG TTCAAAATGG  540
CCCATCCCAA GAAGAGCTGG AAATCCAGAG AAGGCAACTG CAAGAACAGC AGCGACAGAA  600
GGAACTGGAG AGGGAAAGAA TGGAGAGGGA AAGGTTGGAG AGAGAACGAC TAGAACGAGA  660
GAGGCTAGAG AGGGAGCGCC TGGAACAAGA GCAGCTGGAG CGGCAGCGGC AGGAAAGGGA  720
GCACGTGGAG CGGCTGGAGA GGGAGAGGCT GGAGCGCCTG GAGCGAGAGA GGCAGGAGCG  780
GGAGCGAGAG CGCCTGGAGC AGCTGGAGCG GGAGCAAGTG GAGTGGGAGC GAGAGCGCAG  840
AATGTCCAAT GCTGCTCCAT CTTCAGACAG CTCCCTGTCT AGTGCTCCAC TTCCTGAGTA  900
TTCCAGTTGC CAGCCGCCTT CGGCACCTCC TCCATCATAT GCTAAAGTCA TCTCAGCTCC  960
GGTGTCAGAC GCCACTCCTG ATTACGCTGT AGTGACTGCT TTGCCACCTA CTTCCACACC 1020
CCCTACACCA CCACTGAGAC ACGCAGCGAC ACGTTTTGCA ACATCTCTAG GTTCAGCCTT 1080
CCACCCTGTT CTTCCCCATT ACGCTACAGT TCCTCGTCCT CTCAACAAAA ACTCTCGACC 1140
TTCTTCTCCT GTGAACACAC CCTCTTCTCA GCCTCCAGCT GCGAAGTCCT GTGCCTGGCC 1200
```

TACTTCCAAT TTCTCGCCCC TCCCTCCATC TCCTCCAATA ATGATTAGCA GCCCCCCTGG 1260
CAAAGCTACT GGNCCACGGC CTGTCCTTCC CGTTTGTGTC TCCTCTCCTG TGCCCCAAAT 1320
GCCTCCGTCA CCAACAGCAC CCAATGGGTC GCTAGACTCT GTAACATACC CAGTGTCTCC 1380
ACCGCCTACC TCAGGGCCAG CAGCGCCACC TCCGCCGCCA CCGCCACCGC CGCCGCCACC 1440
ACCACCGCCG CTGCCACCGC CGCCGCTGCC TCCCCTCGCC TCACTCTCAC ACTGTGGATC 1500
ACAGGCTTCT CCTCCTCCAG GCACCCCTCT TGCCTCAACT CCCTCATCCA AGCCCAGTGT 1560
TCTCCCTTCT CCCTCTGCAG GTGCCCCTGC CTCTGCGGAG ACCCCTCTAA ATCCTGAGCT 1620
GGGAGACTCC TCTGCTTCCG AGCCAGGCTT GCAGGCAGCC TCTCAGCCGG CCGAGTCGCC 1680
AACCCCACAG GGCCTTGTCT TGGGACCACC TGCACCTCCG CCACCACCCC CTCTCCCATC 1740
AGGCCCTGCC TACGCCTCAG CACTTCCTCC TCCCCCAGGA CCCCCTCCAC CACCTCCACT 1800
GCCATCCACT GGTCCTCCTC CTCCACCCCC TCCACCACCC CCTCTTCCTA ATCAAGCTCC 1860
TCCCCCTCCT CCCCCACCTC CTGCCCCTCC CCTCCCCGCA TCTGGAATTT TCTCTGGATC 1920
CACGTCAGAA GACAATCGCC CTTTAACTGG ACTTGCAGCT GCAATTGCGG GAGCAAAACT 1980
TAGGAAAGTG TCCCGGGTGG AGGATGGCTC TTTCCCAGGT GGAGGGAATA CTGGGAGTGT 2040
GAGCTTGGCC TCATCCAAAG CAGACGCTGG GCGTGGGAAT GGACCTCTTC CTCTAGGGGG 2100
TAGTGGCTTA ATGGAAGAAA TGAGTGCCCT GCTGGCCAGG AGGAGAAGAA TTGCTGAGAA 2160
GGGATCAACA ATAGAAACAG AACAAAAGGA AGACAGAAAT GAAGATGCAG AGCCTATAAC 2220
TGCTAAGGCC CCATCAACAA GTACACCTGA ACCAACCAGA AAACCTTGGG AAAGAACAAA 2280
CACAATGAAC GGCAGTAAGT CACCTGTCAT CTCCAGACCC AAATCCACAC CTTCATCACA 2340
GCCAAGTGCC AATGGAGTCC AGACAGAAGG CCTTGACTAT GACAGGCTGA AGCAGGACAT 2400
TTTAGATGAG ATGAGAAAAG AGCTGGCAAA GCTGAAGGAG GAGCTTATTG ACGCAATCAG 2460
GCAGGAGCTG AGCAAGTCGA ACACTGCATA AAGAAGCAAA CTACGGAGGG GCAGGACTTG 2520
AATCTGGAGA AAACAAAAAT TCCTACAAAC AACTCTTAAC CCCCAAACTT TTAAGCTGTA 2580
AGAAGAAAAT GGATACACAG TCAGGAGGGA AGCCGTCAAC CTCTGAAAGC CTCAGACAGT 2640
GACTCTGGCG ATCAGCTGTC CCCTCAGTGT GCTGCTTTAT TCTGTCTGAC CTTTACCACA 2700
GGATGGAGAA TGATATTGGA GTTCCCTTAG CAGTACTAAA CCCGTCAGGC AAGATCACCG 2760
TGCATTGAAA TATTTTCATG TCTAGATGAG TCTGCACGTT TTCCATAATC CATTGCTAAA 2820
ATAAAGACGA GAAAGGGTAA ATCTCGAGGA ATTCATAATT TTTTCCTCCA GATCCTCTAG 2880
AGTCCTGTTT CCTGTGTG 2898

(2) INFORMATION FOR SEQ ID NO.4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 783 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: Neural Mena+ Deduced Amino Acid Sequence

(ix) FEATURE:
(A) NAME/KEY: Region
(B) LOCATION: 239..482
(D) OTHER INFORMATION: /label= insertion
/note= "Inserted amino acids common to neural Mena+, neural Mena++, and neural Mena+++"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO.4:

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Met Ser Glu Gln Ser Ile Cys Gln Ala Arg Ala Ala Val Met Val Tyr
1               5                   10                  15

Asp Asp Ala Asn Lys Lys Trp Val Pro Ala Gly Gly Ser Thr Gly Phe
            20                  25                  30

Ser Arg Val His Ile Tyr His His Thr Gly Asn Asn Thr Phe Arg Val
        35                  40                  45

Val Gly Arg Lys Ile Gln Asp His Gln Val Val Ile Asn Cys Ala Ile
    50                  55                  60

Pro Lys Gly Leu Lys Tyr Asn Gln Ala Thr Gln Thr Phe His Gln Trp
65                  70                  75                  80

Arg Asp Ala Arg Gln Val Tyr Gly Leu Asn Phe Gly Ser Lys Glu Asp
                85                  90                  95

Ala Asn Val Phe Ala Ser Ala Met Met His Ala Leu Glu Val Leu Asn
            100                 105                 110

Ser Gln Glu Ala Gly Pro Thr Leu Pro Arg Gln Asn Ser Gln Leu Pro
        115                 120                 125

Ala Gln Val Gln Asn Gly Pro Ser Gln Glu Glu Leu Glu Ile Gln Arg
    130                 135                 140

Arg Gln Leu Gln Glu Gln Gln Arg Gln Lys Glu Leu Glu Arg Glu Arg
145                 150                 155                 160

Met Glu Arg Glu Arg Leu Glu Arg Glu Arg Leu Glu Arg Glu Arg Leu
                165                 170                 175

Glu Arg Glu Arg Leu Glu Gln Glu Gln Leu Glu Arg Gln Arg Gln Glu
            180                 185                 190

Arg Glu His Val Glu Arg Leu Glu Arg Glu Arg Leu Glu Arg Leu Glu
        195                 200                 205

Arg Glu Arg Gln Glu Arg Glu Arg Glu Arg Leu Glu Gln Leu Glu Arg
    210                 215                 220

Glu Gln Val Glu Trp Glu Arg Glu Arg Arg Met Ser Asn Ala Ala Pro
225                 230                 235                 240

Ser Ser Asp Ser Ser Leu Ser Ser Ala Pro Leu Pro Glu Tyr Ser Ser
                245                 250                 255

Cys Gln Pro Pro Ser Ala Pro Pro Pro Ser Tyr Ala Lys Val Ile Ser
            260                 265                 270

Ala Pro Val Ser Asp Ala Thr Pro Asp Tyr Ala Val Val Thr Ala Leu
        275                 280                 285
```

Pro Pro Thr Ser Thr Pro Pro Thr Pro Pro Leu Arg His Ala Ala Thr
290 295 300

Arg Phe Ala Thr Ser Leu Gly Ser Ala Phe His Pro Val Leu Pro His
305 310 315 320

Tyr Ala Thr Val Pro Arg Pro Leu Asn Lys Asn Ser Arg Pro Ser Ser
325 330 335

Pro Val Asn Thr Pro Ser Ser Gln Pro Pro Ala Ala Lys Ser Cys Ala
340 345 350

Trp Pro Thr Ser Asn Phe Ser Pro Leu Pro Pro Ser Pro Pro Ile Met
355 360 365

Ile Ser Ser Pro Pro Gly Lys Ala Thr Gly Pro Arg Pro Val Leu Pro
370 375 380

Val Cys Val Ser Ser Pro Val Pro Gln Met Pro Pro Ser Pro Thr Ala
385 390 395 400

Pro Asn Gly Ser Leu Asp Ser Val Thr Tyr Pro Val Ser Pro Pro Pro
405 410 415

Thr Ser Gly Pro Ala Ala Pro Pro Pro Pro Pro Pro Pro Pro Pro Pro
420 425 430

Pro Pro Pro Pro Pro Leu Pro Pro Pro Pro Leu Pro Pro Leu Ala Ser
435 440 445

Leu Ser His Cys Gly Ser Gln Ala Ser Pro Pro Pro Gly Thr Pro Leu
450 455 460

Ala Ser Thr Pro Ser Ser Lys Pro Ser Val Leu Pro Ser Pro Ser Ala
465 470 475 480

Gly Ala Pro Ala Ser Ala Glu Thr Pro Leu Asn Pro Glu Leu Gly Asp
485 490 495

Ser Ser Ala Ser Glu Pro Gly Leu Gln Ala Ala Ser Gln Pro Ala Glu
500 505 510

Ser Pro Thr Pro Gln Gly Leu Val Leu Gly Pro Pro Ala Pro Pro Pro
515 520 525

Pro Pro Pro Leu Pro Ser Gly Pro Ala Tyr Ala Ser Ala Leu Pro Pro
530 535 540

Pro Pro Gly Pro Pro Pro Pro Pro Pro Leu Pro Ser Thr Gly Pro Pro
545 550 555 560

Pro Pro Pro Pro Pro Pro Pro Pro Leu Pro Asn Gln Ala Pro Pro Pro
565 570 575

Pro Pro Pro Pro Pro Ala Pro Pro Leu Pro Ala Ser Gly Ile Phe Ser
580 585 590

Gly Ser Thr Ser Glu Asp Asn Arg Pro Leu Thr Gly Leu Ala Ala Ala
595 600 605

Ile Ala Gly Ala Lys Leu Arg Lys Val Ser Arg Val Glu Asp Gly Ser
610 615 620

Phe Pro Gly Gly Gly Asn Thr Gly Ser Val Ser Leu Ala Ser Ser Lys
625 630 635 640

Ala Asp Ala Gly Arg Gly Asn Gly Pro Leu Pro Leu Gly Gly Ser Gly
645 650 655

Leu Met Glu Glu Met Ser Ala Leu Leu Ala Arg Arg Arg Arg Ile Ala
660 665 670

Glu Lys Gly Ser Thr Ile Glu Thr Glu Gln Lys Glu Asp Arg Asn Glu
675 680 685

Asp Ala Glu Pro Ile Thr Ala Lys Ala Pro Ser Thr Ser Thr Pro Glu
690 695 700

Pro Thr Arg Lys Pro Trp Glu Arg Thr Asn Thr Met Asn Gly Ser Lys
705 710 715 720

Ser Pro Val Ile Ser Arg Pro Lys Ser Thr Pro Ser Ser Gln Pro Ser
725 730 735

Ala Asn Gly Val Gln Thr Glu Gly Leu Asp Tyr Asp Arg Leu Lys Gln
740 745 750

Asp Ile Leu Asp Glu Met Arg Lys Glu Leu Ala Lys Leu Lys Glu Glu
755 760 765

Leu Ile Asp Ala Ile Arg Gln Glu Leu Ser Lys Ser Asn Thr Ala
770 775 780

(2) INFORMATION FOR SEQ ID NO.S:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 787 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: Neural Mena++

(ix) FEATURE:
(A) NAME/KEY: Region
(B) LOCATION: 117..119
(D) OTHER INFORMATION: /label= Insertion
/note= "Inserted amino acids in neural Mena++"

(ix) FEATURE:
(A) NAME/KEY: Region
(B) LOCATION: 239..482
(D) OTHER INFORMATION: /label= insertion
/note= "Inserted amino acids common to neural Mena+, neural Mena++ and Neural Mena+++"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 5:

Met Ser Glu Gln Ser Ile Cys Gln Ala Arg Ala Ala Val Met Val Tyr
1 5 10 15

Asp Asp Ala Asn Lys Lys Trp Val Pro Ala Gly Gly Ser Thr Gly Phe
20 25 30

Ser Arg Val His Ile Tyr His His Thr Gly Asn Asn Thr Phe Arg Val
35 40 45

Val Gly Arg Lys Ile Gln Asp His Gln Val Val Ile Asn Cys Ala Ile
50 55 60

Pro Lys Gly Leu Lys Tyr Asn Gln Ala Thr Gln Thr Phe His Gln Trp
65 70 75 80

Arg Asp Ala Arg Gln Val Tyr Gly Leu Asn Phe Gly Ser Lys Glu Asp
85 90 95

Ala Asn Val Phe Ala Ser Ala Met Met His Ala Leu Glu Val Leu Asn
100 105 110

Ser Gln Glu Ala Val Phe Tyr Leu Gly Pro Thr Leu Pro Arg Gln Asn
115 120 125

Ser Gln Leu Pro Ala Gln Val Gln Asn Gly Pro Ser Gln Glu Glu Leu
130 135 140

Glu Ile Gln Arg Arg Gln Leu Gln Glu Gln Gln Arg Gln Lys Glu Leu
145 150 155 160

Glu Arg Glu Arg Met Glu Arg Glu Arg Leu Glu Arg Glu Arg Leu Glu
165 170 175

Arg Glu Arg Leu Glu Arg Glu Arg Leu Glu Gln Glu Gln Leu Glu Arg
180 185 190

Gln Arg Gln Glu Arg Glu His Val Glu Arg Leu Glu Arg Glu Arg Leu
195 200 205

Glu Arg Leu Glu Arg Glu Arg Gln Glu Arg Glu Arg Glu Arg Leu Glu
210 215 220

Gln Leu Glu Arg Glu Gln Val Glu Trp Glu Arg Glu Arg Arg Met Ser
225 230 235 240

Asn Ala Ala Pro Ser Ser Asp Ser Ser Leu Ser Ser Ala Pro Leu Pro
245 250 255

Glu Tyr Ser Ser Cys Gln Pro Pro Ser Ala Pro Pro Pro Ser Tyr Ala
260 265 270

Lys Val Ile Ser Ala Pro Val Ser Asp Ala Thr Pro Asp Tyr Ala Val
275 280 285

Val Thr Ala Leu Pro Pro Thr Ser Thr Pro Pro Thr Pro Pro Leu Arg
290 295 300

His Ala Ala Thr Arg Phe Ala Thr Ser Leu Gly Ser Ala Phe His Pro
305 310 315 320

Val Leu Pro His Tyr Ala Thr Val Pro Arg Pro Leu Asn Lys Asn Ser
325 330 335

Arg Pro Ser Ser Pro Val Asn Thr Pro Ser Ser Gln Pro Pro Ala Ala
340 345 350

Lys Ser Cys Ala Trp Pro Thr Ser Asn Phe Ser Pro Leu Pro Pro Ser
355 360 365

Pro Pro Ile Met Ile Ser Ser Pro Pro Gly Lys Ala Thr Gly Pro Arg
370 375 380

Pro Val Leu Pro Val Cys Val Ser Ser Pro Val Pro Gln Met Pro Pro
385 390 395 400

Ser Pro Thr Ala Pro Asn Gly Ser Leu Asp Ser Val Thr Tyr Pro Val
405 410 415

```
Ser Pro Pro Pro Thr Ser Gly Pro Ala Ala Pro Pro Pro Pro Pro Pro
            420                 425                 430

Pro Pro Pro Pro Pro Pro Pro Pro Pro Leu Pro Pro Pro Pro Leu Pro
        435                 440                 445

Pro Leu Ala Ser Leu Ser His Cys Gly Ser Gln Ala Ser Pro Pro Pro
    450                 455                 460

Gly Thr Pro Leu Ala Ser Thr Pro Ser Ser Lys Pro Ser Val Leu Pro
465                 470                 475                 480

Ser Pro Ser Ala Gly Ala Pro Ala Ser Ala Glu Thr Pro Leu Asn Pro
                485                 490                 495

Glu Leu Gly Asp Ser Ser Ala Ser Glu Pro Gly Leu Gln Ala Ala Ser
            500                 505                 510

Gln Pro Ala Glu Ser Pro Thr Pro Gln Gly Leu Val Leu Gly Pro Pro
        515                 520                 525

Ala Pro Pro Pro Pro Pro Pro Leu Pro Ser Gly Pro Ala Tyr Ala Ser
    530                 535                 540

Ala Leu Pro Pro Pro Pro Gly Pro Pro Pro Pro Pro Pro Leu Pro Ser
545                 550                 555                 560

Thr Gly Pro Pro Pro Pro Pro Pro Pro Pro Pro Pro Leu Pro Asn Gln
                565                 570                 575

Ala Pro Pro Pro Pro Pro Pro Pro Pro Ala Pro Pro Leu Pro Ala Ser
            580                 585                 590

Gly Ile Phe Ser Gly Ser Thr Ser Glu Asp Asn Arg Pro Leu Thr Gly
        595                 600                 605

Leu Ala Ala Ala Ile Ala Gly Ala Lys Leu Arg Lys Val Ser Arg Val
    610                 615                 620

Glu Asp Gly Ser Phe Pro Gly Gly Gly Asn Thr Gly Ser Val Ser Leu
625                 630                 635                 640

Ala Ser Ser Lys Ala Asp Ala Gly Arg Gly Asn Gly Pro Leu Pro Leu
                645                 650                 655

Gly Gly Ser Gly Leu Met Glu Glu Met Ser Ala Leu Leu Ala Arg Arg
            660                 665                 670

Arg Arg Ile Ala Glu Lys Gly Ser Thr Ile Glu Thr Glu Gln Lys Glu
        675                 680                 685

Asp Arg Asn Glu Asp Ala Glu Pro Ile Thr Ala Lys Ala Pro Ser Thr
    690                 695                 700

Ser Thr Pro Glu Pro Thr Arg Lys Pro Trp Glu Arg Thr Asn Thr Met
705                 710                 715                 720

Asn Gly Ser Lys Ser Pro Val Ile Ser Arg Pro Lys Ser Thr Pro Ser
                725                 730                 735

Ser Gln Pro Ser Ala Asn Gly Val Gln Thr Glu Gly Leu Asp Tyr Asp
            740                 745                 750

Arg Leu Lys Gln Asp Ile Leu Asp Glu Met Arg Lys Glu Leu Ala Lys
        755                 760                 765
```

```
Leu Lys Glu Glu Leu Ile Asp Ala Ile Arg Gln Glu Leu Ser Lys Ser
    770                 775                 780

Asn Thr Ala
785
```

(2) INFORMATION FOR SEQ ID NO. 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 802 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: Neural Mena+++

(ix) FEATURE:
(A) NAME/KEY: Region
(B) LOCATION: 117..135
(D) OTHER INFORMATION: /label= Insertion
/note= "Inserted amino acids in neural Mena Isoform 2"

(ix) FEATURE:
(A) NAME/KEY: Region
(B) LOCATION: 239..482
(D) OTHER INFORMATION: /label= insertion
/note= "Inserted amino acids common to neural Mena+, neural Mena++ and neural Mena+++"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 6:

```
Met Ser Glu Gln Ser Ile Cys Gln Ala Arg Ala Ala Val Met Val Tyr
1               5                   10                  15

Asp Asp Ala Asn Lys Lys Trp Val Pro Ala Gly Gly Ser Thr Gly Phe
            20                  25                  30

Ser Arg Val His Ile Tyr His His Thr Gly Asn Asn Thr Phe Arg Val
        35                  40                  45

Val Gly Arg Lys Ile Gln Asp His Gln Val Val Ile Asn Cys Ala Ile
    50                  55                  60

Pro Lys Gly Leu Lys Tyr Asn Gln Ala Thr Gln Thr Phe His Gln Trp
65                  70                  75                  80

Arg Asp Ala Arg Gln Val Tyr Gly Leu Asn Phe Gly Ser Lys Glu Asp
                85                  90                  95

Ala Asn Val Phe Ala Ser Ala Met Met His Ala Leu Glu Val Leu Asn
            100                 105                 110

Ser Gln Glu Ala Ala Gln Ser Lys Val Thr Ala Thr Gln Asp Ser Thr
        115                 120                 125

Asn Leu Arg Cys Ile Phe Cys Gly Pro Thr Leu Pro Arg Gln Asn Ser
    130                 135                 140

Gln Leu Pro Ala Gln Val Gln Asn Gly Pro Ser Gln Glu Glu Leu Glu
145                 150                 155                 160
```

```
Ile Gln Arg Arg Gln Leu Gln Glu Gln Gln Arg Gln Lys Glu Leu Glu
                165                 170                 175

Arg Glu Arg Met Glu Arg Glu Arg Leu Glu Arg Glu Arg Leu Glu Arg
            180                 185                 190

Glu Arg Leu Glu Arg Glu Arg Leu Glu Gln Glu Gln Leu Glu Arg Gln
        195                 200                 205

Arg Gln Glu Arg Glu His Val Glu Arg Leu Glu Arg Glu Arg Leu Glu
    210                 215                 220

Arg Leu Glu Arg Glu Arg Gln Glu Arg Glu Arg Glu Arg Leu Glu Gln
225                 230                 235                 240

Leu Glu Arg Glu Gln Val Glu Trp Glu Arg Glu Arg Arg Met Ser Asn
                245                 250                 255

Ala Ala Pro Ser Ser Asp Ser Ser Leu Ser Ser Ala Pro Leu Pro Glu
            260                 265                 270

Tyr Ser Ser Cys Gln Pro Pro Ser Ala Pro Pro Pro Ser Tyr Ala Lys
        275                 280                 285

Val Ile Ser Ala Pro Val Ser Asp Ala Thr Pro Asp Tyr Ala Val Val
    290                 295                 300

Thr Ala Leu Pro Pro Thr Ser Thr Pro Pro Thr Pro Pro Leu Arg His
305                 310                 315                 320

Ala Ala Thr Arg Phe Ala Thr Ser Leu Gly Ser Ala Phe His Pro Val
                325                 330                 335

Leu Pro His Tyr Ala Thr Val Pro Arg Pro Leu Asn Lys Asn Ser Arg
            340                 345                 350

Pro Ser Ser Pro Val Asn Thr Pro Ser Ser Gln Pro Pro Ala Ala Lys
        355                 360                 365

Ser Cys Ala Trp Pro Thr Ser Asn Phe Ser Pro Leu Pro Pro Ser Pro
    370                 375                 380

Pro Ile Met Ile Ser Ser Pro Pro Gly Lys Ala Thr Gly Pro Arg Pro
385                 390                 395                 400

Val Leu Pro Val Cys Val Ser Ser Pro Val Pro Gln Met Pro Pro Ser
                405                 410                 415

Pro Thr Ala Pro Asn Gly Ser Leu Asp Ser Val Thr Tyr Pro Val Ser
            420                 425                 430

Pro Pro Pro Thr Ser Gly Pro Ala Ala Pro Pro Pro Pro Pro Pro Pro
        435                 440                 445

Pro Pro Pro Pro Pro Pro Pro Pro Leu Pro Pro Pro Pro Leu Pro Pro
    450                 455                 460

Leu Ala Ser Leu Ser His Cys Gly Ser Gln Ala Ser Pro Pro Pro Gly
465                 470                 475                 480

Thr Pro Leu Ala Ser Thr Pro Ser Ser Lys Pro Ser Val Leu Pro Ser
                485                 490                 495

Pro Ser Ala Gly Ala Pro Ala Ser Ala Glu Thr Pro Leu Asn Pro Glu
            500                 505                 510
```

Leu Gly Asp Ser Ser Ala Ser Glu Pro Gly Leu Gln Ala Ala Ser Gln
515 520 525

Pro Ala Glu Ser Pro Thr Pro Gln Gly Leu Val Leu Gly Pro Pro Ala
530 535 540

Pro Pro Pro Pro Pro Pro Leu Pro Ser Gly Pro Ala Tyr Ala Ser Ala
545 550 555 560

Leu Pro Pro Pro Pro Gly Pro Pro Pro Pro Pro Pro Leu Pro Ser Thr
565 570 575

Gly Pro Pro Pro Pro Pro Pro Pro Pro Pro Pro Leu Pro Asn Gln Ala
580 585 590

Pro Pro Pro Pro Pro Pro Pro Pro Ala Pro Pro Leu Pro Ala Ser Gly
595 600 605

Ile Phe Ser Gly Ser Thr Ser Glu Asp Asn Arg Pro Leu Thr Gly Leu
610 615 620

Ala Ala Ala Ile Ala Gly Ala Lys Leu Arg Lys Val Ser Arg Val Glu
625 630 635 640

Asp Gly Ser Phe Pro Gly Gly Gly Asn Thr Gly Ser Val Ser Leu Ala
645 650 655

Ser Ser Lys Ala Asp Ala Gly Arg Gly Asn Gly Pro Leu Pro Leu Gly
660 665 670

Gly Ser Gly Leu Met Glu Glu Met Ser Ala Leu Leu Ala Arg Arg Arg
675 680 685

Arg Ile Ala Glu Lys Gly Ser Thr Ile Glu Thr Glu Gln Lys Glu Asp
690 695 700

Arg Asn Glu Asp Ala Glu Pro Ile Thr Ala Lys Ala Pro Ser Thr Ser
705 710 715 720

Thr Pro Glu Pro Thr Arg Lys Pro Trp Glu Arg Thr Asn Thr Met Asn
725 730 735

Gly Ser Lys Ser Pro Val Ile Ser Arg Pro Lys Ser Thr Pro Ser Ser
740 745 750

Gln Pro Ser Ala Asn Gly Val Gln Thr Glu Gly Leu Asp Tyr Asp Arg
755 760 765

Leu Lys Gln Asp Ile Leu Asp Glu Met Arg Lys Glu Leu Ala Lys Leu
770 775 780

Lys Glu Glu Leu Ile Asp Ala Ile Arg Gln Glu Leu Ser Lys Ser Asn
785 790 795 800

Thr Ala

(2) INFORMATION FOR SEQ ID NO. 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1956 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mus musculus

(vii) IMMEDIATE SOURCE:
(B) CLONE: Evl

(ix) FEATURE:
(A) NAME/KEY: CDS

(B) LOCATION: 418..1599

(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 7:

```
AGCTGGCGAA AGGGGATGTG CTGCAAGGCG ATTAAGTTGG TAACGCCAGG TTTTCCCAGT   60
CANGACGTTG TAAAACGACG GCCAGTGAAT TGTAATACGA CTCACTATAG GGCGAATTGG  120
TACCGGGCCC CCCTTCGAGG TCGACGGTAT CGATAAGCTT GATATGCGCC GGCCCGGGCA  180
CGCGCGCCCG CCAAGATGGC AGGGGCCGGG GCCCAGNTGT CAGTCGCCAC CGCCGCCGCC  240
GCGGCGGCCG CGTTGCTTCG CAGCTAGCGG CCCGGACGCC CGCCGGAGAA GATGAGCCCC  300
CGCCGCCGCN TGCAGCCCAG CCAGACGCGG AGCCGCCGCG CCCGGGGTCG GCTGCCCCGC  360
TAGCGTCCCG TCGCGCCGCG CTTTCATCCC CGGCCCCGTG CCCGCCCGGC AGCCACGATG  420
AGTGAACAGA GTATCTGCCA AGCGCGGGCC TCCGTGATGG TCTACGATGA CACCAGTAAG  480
AAGTGGGTAC CGATCAAGCC TGGCCAGCAG GGATTCAGCC GGATCAACAT CTACCACAAC  540
ACTGCCAGCA GCACCTTCAG AGTGGTCGGG GTCAAGCTAC AGGACCAGCA GGTTGTGATC  600
AATTATTCAA TTGTTAAAGG GCTGAAGTAC AATCAGGCAA CACCCACCTT CCATCAGTGG  660
CGAGATGCCC GTCAGGTCTA TGGCTTAAAC TTTGCAAGTA AGGAAGAAGC AACCACATTC  720
TCCAATGCCA TGCTCTTTGC CCTGAACATC ATGAATTCCC AAGAAGGAGG CCCCTCCACA  780
CAGCGTCAGG TGCAGAATGG CCCCTCTCCT GAGGAGATGG ACATCCAGAG AAGACAAGTA  840
ATGGAGCAGC AGCACCGCCA GGAGTCTCTG GAGAGGAGAA TCTCGGCCAC AGGGCCCATT  900
CTCCCCCCTG GGCATCCCTC ATCGGCAGCC AGCACCACTC TCTCCTGTAG TGGACCTCCA  960
CCCCCGCCTC CACCCCCAGT TCCACCTCCA CCCACAGGGT CTACTCCCCC ACCCCCACCC 1020
CCACTGCCAG CTGGAGGAGC CCAGGGGACC AACCATGATG.AGAGCTCTGC ATCAGGACTG 1080
GCTGCTGCTC TGGCGGGAGC CAAGCTAAGG AGGGTGCAGC GGCCAGAAGA TGCATCTGGA 1140
GGCTCCAGTC CTAGTGGGAC TTCAAAGTCC GATGCCAACC GGGCAAGCAG TGGGGGAGGT 1200
GGAGGAGGCC TCATGGAAGA AATGAACAAG CTGCTGGCTA AGAGGAGAAA GGCAGCCTCC 1260
CAGACAGACA AGCCCGCTGA CAGAAAGGAA GATGAGAGCC AAACGGAAGA CCCTAGCACC 1320
TCCCCATCCC CAGGTACCCG AGCCACCAGC CAGCCACCTA ATTCCTCAGA GGCTGGCAGA 1380
AAACCCTGGG AACGGAGCAA CTCGGTGGAG AAACCTGTGT CCTCGTTGCT GTCCAGGGTG 1440
AAGCCTGCTG GGAGTGTGAA TGACGTGGGC CTGGATGCCT TAGATTTGGA CCGGATGAAA 1500
CAGGAGATCC TGGAGGAGGT GGTTCGGGAG CTGCACAAGG TGAAGGAGGA GATCATTGAT 1560
GCCATCAGGC AGGAGCTAAG TGGGATCAGC ACCACGTAAG ATGGCACCAG TCCTGGAGGA 1620
```

TTGCGAGGAG CCGTGCTGGC CCCAGCGAGC ATCGAGCCTG CAGAAGCTGG CATGTACTTA 1680

AGTCTCAACC TGTGATACAA TCTTAAAATG AGGAAACAAA CTTCAACTCC TGGATTTTTT 1740

AGTGTATCTG ACACAGAACA CCGGGTCTAT TCTTTTTTTG TATTTTATAT TTGCTTATTT 1800

AAGTGTACGT TCCTTTGGTT TATAGAGAAC ACCCCCAAAT CACCTGCTTT ATTAGATGGC 1860

TTCCAAGTTT TCTCCTAGGT GACACTGTTG GTGCCTCAGC TGACAGGGAG CAGCTGGGTG 1920

CAGTGTGGCC TTTCCATGCC ACAGAGCTGT CAGAAT 1956

(2) INFORMATION FOR SEQ ID NO. 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 393 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: Evl Deduced Amino Acid Sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO.8:

Met Ser Glu Gln Ser Ile Cys Gln Ala Arg Ala Ser Val Met Val Tyr
1 5 10 15

Asp Asp Thr Ser Lys Lys Trp Val Pro Ile Lys Pro Gly Gln Gln Gly
20 25 30

Phe Ser Arg Ile Asn Ile Tyr His Asn Thr Ala Ser Ser Thr Phe Arg
35 40 45

Val Val Gly Val Lys Leu Gln Asp Gln Gln Val Val Ile Asn Tyr Ser
50 55 60

Ile Val Lys Gly Leu Lys Tyr Asn Gln Ala Thr Pro Thr Phe His Gln
65 70 75 80

Trp Arg Asp Ala Arg Gln Val Tyr Gly Leu Asn Phe Ala Ser Lys Glu
85 90 95

Glu Ala Thr Thr Phe Ser Asn Ala Met Leu Phe Ala Leu Asn Ile Met
100 105 110

Asn Ser Gln Glu Gly Gly Pro Ser Thr Gln Arg Gln Val Gln Asn Gly
115 120 125

Pro Ser Pro Glu Glu Met Asp Ile Gln Arg Arg Gln Val Met Glu Gln
130 135 140

Gln His Arg Gln Glu Ser Leu Glu Arg Arg Ile Ser Ala Thr Gly Pro
145 150 155 160

Ile Leu Pro Pro Gly His Pro Ser Ser Ala Ala Ser Thr Thr Leu Ser
165 170 175

Cys Ser Gly Pro Pro Pro Pro Pro Pro Pro Pro Val Pro Pro Pro Pro
180 185 190

Thr Gly Ser Thr Pro Pro Pro Pro Pro Pro Leu Pro Ala Gly Gly Ala
195 200 205

Gln Gly Thr Asn His Asp Glu Ser Ser Ala Ser Gly Leu Ala Ala Ala
210 215 220

Leu Ala Gly Ala Lys Leu Arg Arg Val Gln Arg Pro Glu Asp Ala Ser
225 230 235 240

Gly Gly Ser Ser Pro Ser Gly Thr Ser Lys Ser Asp Ala Asn Arg Ala
245 250 255

Ser Ser Gly Gly Gly Gly Gly Gly Leu Met Glu Glu Met Asn Lys Leu
260 265 270

Leu Ala Lys Arg Arg Lys Ala Ala Ser Gln Thr Asp Lys Pro Ala Asp
275 280 285

Arg Lys Glu Asp Glu Ser Gln Thr Glu Asp Pro Ser Thr Ser Pro Ser
290 295 300

Pro Gly Thr Arg Ala Thr Ser Gln Pro Pro Asn Ser Ser Glu Ala Gly
305 310 315 320

Arg Lys Pro Trp Glu Arg Ser Asn Ser Val Glu Lys Pro Val Ser Ser
325 330 335

Leu Leu Ser Arg Val Lys Pro Ala Gly Ser Val Asn Asp Val Gly Leu
340 345 350

Asp Ala Leu Asp Leu Asp Arg Met Lys Gln Glu Ile Leu Glu Glu Val
355 360 365

Val Arg Glu Leu His Lys Val Lys Glu Glu Ile Ile Asp Ala Ile Arg
370 375 380

Gln Glu Leu Ser Gly Ile Ser Thr Thr
385 390

(2) INFORMATION FOR SEQ ID NO. 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 740 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 9:

Met Ser Glu Gln Ser Ile Cys Xaa Ala Arg Ala Xaa Val Met Val Tyr
1 5 10 15

Asp Asp Xaa Xaa Lys Lys Trp Val Pro Xaa Xaa Xaa Gly Xaa Xaa Gly
20 25 30

Phe Ser Arg Val Xaa Ile Tyr His Xaa Xaa Xaa Xaa Asn Thr Phe Arg
35 40 45

Val Val Gly Arg Lys Leu Gln Xaa Asp Xaa Gln Val Val Ile Asn Cys
50 55 60

Xaa Ile Xaa Lys Gly Leu Lys Tyr Asn Gln Ala Thr Pro Thr Phe His
65 70 75 80

Gln Trp Arg Asp Ala Arg Gln Val Tyr Gly Leu Asn Phe Xaa Ser Lys
85 90 95

Glu Asp Ala Xaa Xaa Phe Ala Xaa Ala Met Xaa Xaa Ala Leu Glu Xaa
100 105 110

Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Gly Xaa Pro Xaa Xaa Xaa
115 120 125

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Val Xaa Asn Gly Pro Ser
130 135 140

Xaa Glu Glu Xaa Xaa Xaa Gln Xaa Arg Gln Xaa Xaa Xaa Xaa Xaa Xaa
145 150 155 160

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
165 170 175

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
180 185 190

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
195 200 205

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
210 215 220

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Glu
225 230 235 240

Arg Arg Xaa Ser Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
245 250 255

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
260 265 270

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
275 280 285

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
290 295 300

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
305 310 315 320

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
325 330 335

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
340 345 350

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
355 360 365

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
370 375 380

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
385 390 395 400

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Gly Pro Pro Ala
405 410 415

Pro Xaa Xaa Xaa Pro Xaa Xaa Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
420 425 430

Xaa Pro Pro Pro Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Gly Pro Pro Pro
435 440 445

Pro Pro Xaa Xaa Xaa Xaa Xaa Gly Pro Pro Pro Xaa Pro Xaa Xaa Pro
450 455 460

Pro Xaa Xaa Xaa Pro Xaa Xaa Xaa Xaa Xaa Xaa Gly Xaa Xaa Pro Pro
465 470 475 480

Pro Ala Pro Pro Leu Pro Ala Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
485 490 495

Gly Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
500 505 510

Xaa Gly Leu Ala Ala Ala Xaa Ala Gly Ala Lys Leu Arg Lys Val Xaa
515 520 525

Xaa Xaa Glu Xaa Ala Ser Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
530 535 540

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
545 550 555 560

Xaa Xaa Gly Gly Xaa Gly Leu Met Glu Glu Met Xaa Xaa Xaa Leu Ala
565 570 575

Arg Arg Arg Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
580 585 590

Lys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Pro Xaa Xaa Xaa Xaa Xaa Xaa
595 600 605

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Glu Xaa Xaa Arg
610 615 620

Xaa Pro Trp Glu Xaa Xaa Asn Thr Xaa Xaa Xaa Xaa Xaa Ser Xaa Xaa
625 630 635 640

Xaa Ser Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
645 650 655

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
660 665 670

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
675 680 685

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asp Leu
690 695 700

Asp Arg Xaa Lys Gln Glu Ile Leu Xaa Glu Xaa Xaa Xaa Glu Leu Xaa
705 710 715 720

Lys Val Lys Glu Glu Ile Ile Asp Ala Ile Xaa Gln Glu Leu Xaa Xaa
725 730 735

Xaa Xaa Xaa Xaa
740

(2) INFORMATION FOR SEQ ID NO. 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 10:

Leu Glu Arg Glu Arg
1 5

(2) INFORMATION FOR SEQ ID NO. 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 11:

Leu Lys Glu Glu Leu Ile Asp Ala Ile Arg Gln Glu Leu Ser Lys Ser
1 5 10 15

Asn Thr Ala

(2) INFORMATION FOR SEQ ID NO. 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 12:

Ser Glu Pro Ser Ser Phe Glu Phe Pro Pro Pro Pro Thr Asp Glu Glu
1 5 10 15

Leu Arg Leu Ala
20

(2) INFORMATION FOR SEQ ID NO. 13:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 13:

Gly Pro Pro Pro Pro Pro
1 5

(2) INFORMATION FOR SEQ ID NO. 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:
(B) CLONE: MF

(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 14:

AATCGCACAC TCTGTCCATA TTCC 24

(2) INFORMATION FOR SEQ ID NO.15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:
(B) CLONE: MR

(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 15:

TGCCCACAAC TCTGAATGTG TTG 23

(2) INFORMATION FOR SEQ ID NO. 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:
(B) CLONE: BPAF

(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 16:

TCCCAGTCAC GACGTTGTAA AAC 23

(2) INFORMATION FOR SEQ ID NO. 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 17:

Phe Pro Pro Pro Pro
1 5

WHAT IS CLAIMED IS:

We claim:

I . A screening method for detecting the presence in a test sample of a modulator of activity or expression of a *Mena* protein or peptide, comprising the steps of:

providing a control sample including suitable amounts of *Mena* protein or peptide and a *Mena* binding partner under conditions that permit formation of complexes between the binding partner and the *Mena* protein or peptide;

providing a test sample including a test substance and suitable amounts of *Mena* protein or peptide and a selected *Mena*-binding partner under conditions that permit formation of complexes between the binding partner and the *Mena* protein or peptide in the absence of the test substance;

detecting complexes of *Mena* and *Mena*-binding partner in the test sample and in a control sample to determine whether the test substance is a modulator of activity or expression of the *Mena* protein or peptide.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/11669

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) : GOIN 33/53
US CL : 435/7.2
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/7.2; 530/350

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
APS, DIALOG (medline, dissertation abstracts, biosis, embase, derwent, life sciences,conference papers, cancerlit, CAS)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	GERTLER, F.B., "MENA, A RELATIVE OF VASP AND DROSOPHILA ENABLED, IS IMPLICATED IN THE CONTROL OF MICROFILAMNET DYNAMICS", CELL. 18 OCTOBER 1996. VOL 87. NO. 2. PAGES 227-239, SEE ENTIRE DOCUMENT.	1
A	ODDE, D.J., "KINETICS OF MICROTUBULE CATASTROPHE ASSESSED BY PROBABILISTIC ANALYSIS", BIOPHYS J, SEPTEMBER 1995, VOL 69. NO. 3. PAGES 796-802, SEE ENTIRE REFERENCE.	1
A	BLACK, M. M., "MICROTUBULE TRANSPORT AND ASSEMBLY COOPERATE TO GENERATE THE MICROTUBULE ARRAY OF GROWING AXONS", PROG BRAIN RES, 1994, VOL. 102, PAGES 61-77, SEE ENTIRE REFERENCE.	1

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
05 SEPTEMBER 1997	16 OCT 1997

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	SUSAN A. LORING Telephone No. (703) 308-0196